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PREFACE.

In accordance with instructions issued at the commencement of this inquiry, attention has been specially directed towards obtaining facts bearing on the truth or otherwise of two hypotheses regarding the cause of cholera—namely, the theory of its fungoid origin, particularly the one advanced by Professor Hallier of Jena; and the theory of the connection existing between cholera and certain conditions of the soil, promulgated by Professor Max von Pettenkofer of Munich.

In both theories the existence of a specific poison of an organised nature is maintained—a *germ;* and both savants believe it to exist in the alvine discharges of a person affected with cholera. The Munich Professor does not risk an opinion as to whether it belongs to the animal or to the vegetable kingdom, but infers that the soil is the *nidus* in which it grows; whereas Professor Hallier maintains that it multiplies in the human body, and unhesitatingly affirms it to be a fungus.

An account of the observations which have been made in order to test the views advanced by Professor Hallier will occupy the first portion of the report; and, as in the course of the investigation my attention has been directed to a consideration of the microscopic objects which are found in the evacuations of cholera patients, a description of them will at the same time be given; together with illustrations of various initiatory experiments bearing on the general question of 'disease-germs.'

T. R. L.

April 1870.

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	„	
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Fungi developed in ordinary evacuation.

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Blood-cells	lx.	1
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Illustrations of the development of the lower forms of life.

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Segmentation complete	„	5
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⁂ The illustrations are all drawn to scale with the aid of the *camera lucida*, and the magnifying power used is attached to each figure.

The diameter of the object in any of the figures may readily be obtained by comparing them with the one-thousandth of an inch scale placed at the foot of each plate.

These plates have been engraved in the Office of the Surveyor General of India. They are very faithful copies of the original drawings, and will bear favorable comparison with the work of engravers in Europe, who are habitually engaged in this kind of employment. I am under great obligation to the Surveyor General and also to the Assistant Surveyor General, Captain W. G. Murray, under whose immediate superintendence the work has been done.

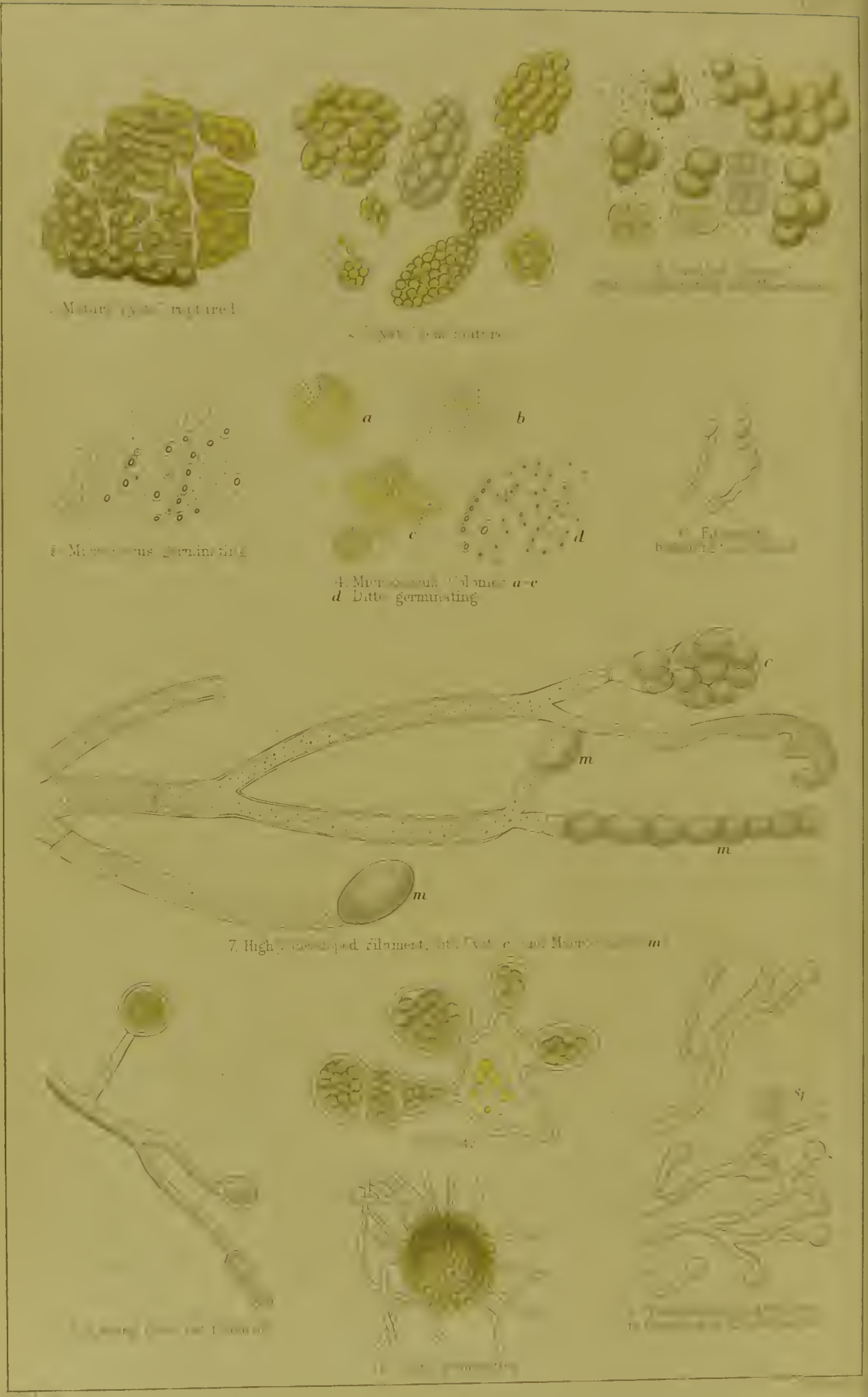

Mature cysts ruptured
a
b
c
d
4. Micrococcus Colonies a–c
d Ditto germinating
c
m
m
m
7. Highly developed filament, with Cysts c and Micrococci m
st

FIG. 1. HALLIER'S DRAWING OF THE "CHOLERA FUNGUS"

INVESTIGATION

INTO THE

MODE OF ORIGIN AND SPREAD OF CHOLERA.

PART I.

CONCERNING THE THEORY OF THE FUNGOID ORIGIN OF CHOLERA AND THE MICROSCOPIC OBJECTS FOUND IN CHOLERAIC EVACUATIONS.

THE theory of the fungoid origin of cholera is based upon the result of certain experiments instituted by Professor Hallier, with the view of ascertaining whether any special organisms could, by means of artificial cultivations, be obtained from choleraic discharges. These experiments have been repeated many times in Calcutta, but as the daily notes of each cultivation would occupy so much space, I propose giving a short summary of a few of the cultivations, illustrated by some of the *camera lucida* drawings which have been accumulated during the investigation. It may, however, previous to doing so, be well to state, in as few words as possible, what the theory really is. As the Professor has published the result of his labours, a short epitome of his *brochure*, weeded of as many technicalities as such a subject will permit; together with a selection of the leading figures in the plate, attached to the book, will, it is thought, best serve to convey his meaning.

Epitome of Hallier's cultivations, compiled from his treatise.*

Some choleraic discharges were sent to the Professor at Jena, obtained from a patient at Berlin during the epidemic of 1866, and another specimen from a patient at Elberfeld during the epidemic in

---

* "Das Cholera Contagium." Von Dr. Ernst Hallier. Leipzig, 1867.

1867. These were examined microscopically and found to contain:—

Discovery of cysts in cholera discharges, which seemed at first sight to present no organised structure.

1. *Cysts* of a yellow or brownish colour, which he for some time believed to be the fructification of *urocystis;* some of these had a very irregular outline, and at first sight seemed to possess no organic structure, caution being necessary not to confound them with masses of fat; application of pressure was, however, found sufficient to discriminate between them. A drawing is given of some of these in a swelled, broken up condition (Fig. i, 1).

Other cysts more distinctly organised.

2. Here and there a few other cysts were seen, considered to be of the same kind as the foregoing; they were spherical or oval cysts varying considerably in size, enclosing a number of yellowish shining spores; the spores also varying in size, as may be seen by a reference to the accompanying figures (Fig. i, 2).

Spores of foregoing in a swelled condition,

3. Groups of swollen gelatinous *spores* surrounded by finely molecular matter (Fig. i, 3). Others appear granular, and some show indication of fission.

which breaking down form "micrococcus."

4. *Micrococcus.*—The molecular matter just alluded to, supposed to have originated from the breaking up of the plasma in the "spore," a little heap often being observed, corresponding to the previously existing spore, called a "micrococcus colony" (Fig. i, 4 *a*), which at *b* is still further broken up; at *c* a group of "colonies" is seen corresponding to the mass of spores previously contained in a cyst whose walls have disappeared. The minute protoplasmic molecules constituting these colonies were seen to adhere to various objects in the fluid, and especially to the particles of epithelium, in fact feeding upon them; this being invariably the way in which vegetable parasites first attack animal tissues. In the midst of these molecules larger ones were observed (Fig. i, 5), which have been figured in a still more advanced stage as torula-like formations at 6.

Action of micrococcus on epithelium.

Development of micrococcus.

This condition being, according to Professor Hallier, the transition stage to the development of the higher forms of fungi.

A series of cultivations was carried out in order to prove

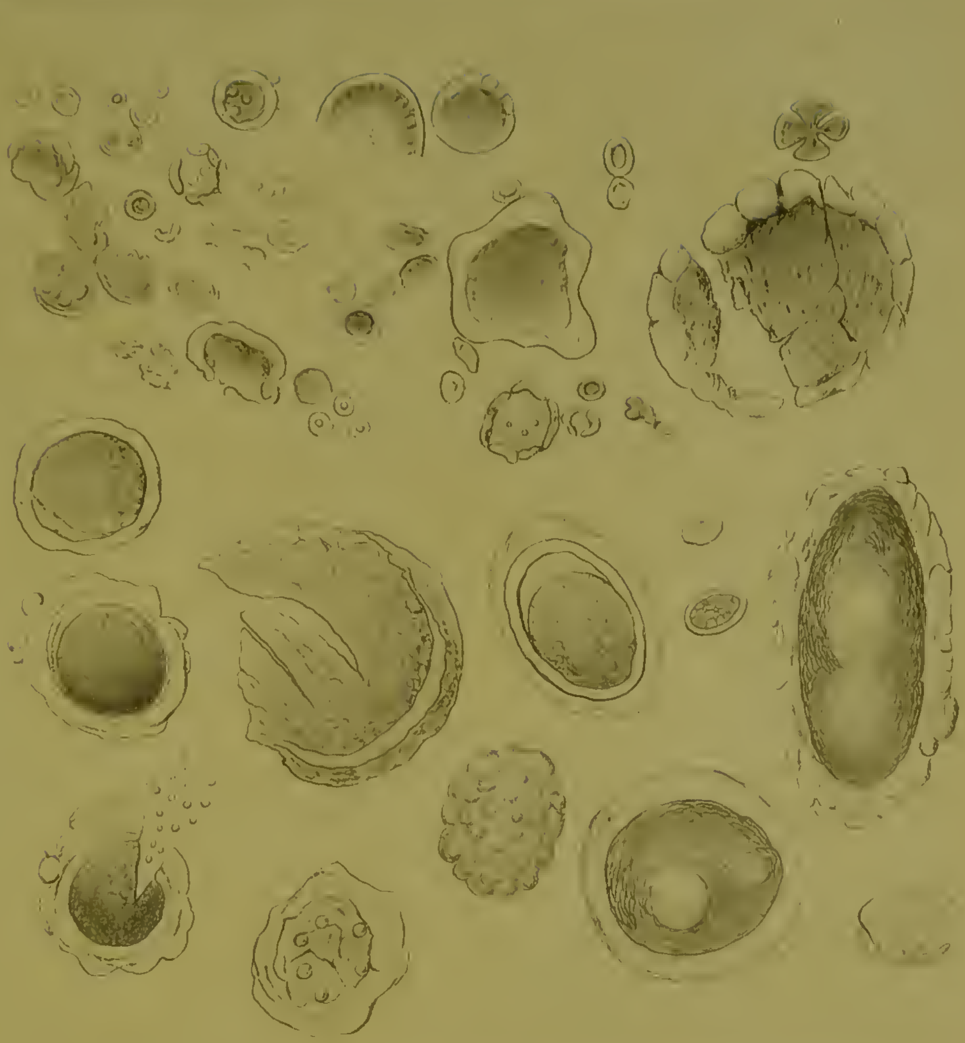
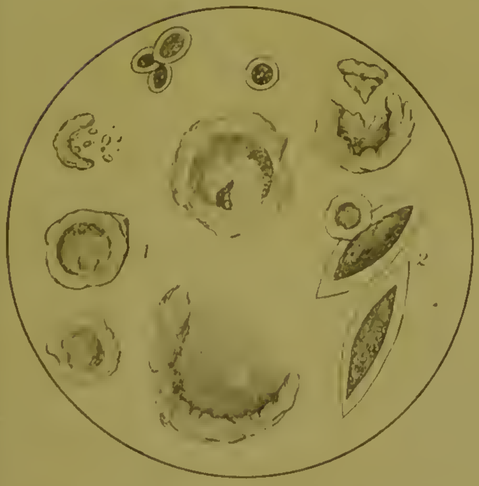


FIG. I. CHOLERA BODIES [illegible]



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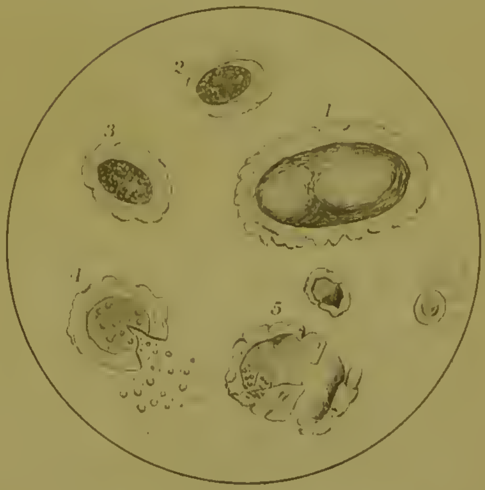

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FIGS. II IV. CHOLERA BODIES OF 1849

Cultivations instituted to prove organic connection between the foregoing.

that these bodies were organically related to each other, namely, that the irregularly defined cysts were advanced stages of the cyst with sharp contour and well marked spore contents; that the circular gelatinous-looking bodies were originally contained in capsules; that the capsules had been borne on a filament; that the filament had originated in a "micrococcus" cell; and that the "micrococcus" had been derived from the disintegration of these gelatinous spores. Portions of the discharges in question were isolated, and placed upon various substrata, beef, starch-paste, slices of lemon, &c., so as to supply the "micrococcus" with other nourishment than the epithelium of the intestinal canal, the disorganization of which substance is, according to Professor Hallier, the prime cause of cholera.

Result of the cultivations.

Germination of micrococcus.

The results of these cultivations may be thus briefly described. During the first two or three days the micrococcus rapidly increased in amount, and developed into nucleated cells, which arranged themselves into chains, as already observed to exist to a slighter extent in the original evacuation. In some cases a thin pellicle formed (*mycoderma*), which, on being lifted, frequently broke down into round balls like the 'micrococcus colonies;' the torula cells about the fourth day were seen to germinate, the ends of the filaments having a linked appearance (6), which continuing to grow, presented the appearance usually seen in *oidium lactis*. In the course of a week the filaments assumed a branched and sacculated appearance (Fig. i, 7, 9), these saccules or joints (termed "macroconidia") being capable of germinating like the spores. The spores were on several occasions seen to produce a peculiar form of fructification, considered by Dr. Hallier to be degenerated *Tilletia caries* (smut), (Fig. i, 9), and on one occasion a spore somewhat like that of smut was detected *(sp.)*; a few abortive attempts at the formation of spore-containing-cysts were also seen. In a few instances, however (about the 9th day), the filaments were observed to bear unmistakable cysts, some with the contained spores very evident (Fig. i, 8), and others in which this condition was less clear.

Development of filaments with formation of macroconidia; some of which simulated the fungus attacking wheat..

The extent of cyst development.

The nearest approach to the development of the cysts, corresponding to those in the discharges, which reminded the Professor so strongly of Urocystis, is figured (Fig. i, 10), and the germination of the same at Fig. i, 11.

Importance attached by Professor Hallier to these cultivations.

The inferences drawn by Dr. Hallier from these experiments in a few words are, that cholera is produced by a species of fungus belonging to the *ustilagines* or smut group. This fungus is a polycystis, similar to that attacking the rye only in Europe, but which the Professor believes attacks the rice plant in India; grounding this belief on the fact that, in the tissue of growing-rice plants watered with choleraic discharges, bodies were detected which he considered identical with the cysts found in the evacuations, thus accounting for the belief frequently expressed by the older writers, that cholera was generated by the consumption of rice in a diseased condition. The author has since modified his views as to the species of fungus in question, but retains the opinion that, whatever the fungus may be called, it closely corresponds with the fungus observed to develop in soil contaminated with choleraic discharges. It will now be seen that Professor Hallier believes that he has established an organic connection between the two kinds of "cysts," "spores" and "micrococcus."

Cysts obtained by inoculating growing-rice plants.

The questions naturally arise—(1) Are there such bodies in the choleraic discharge examined in India? (2) What are they? and (3), are they found under similar circumstances elsewhere?

SECTION I.—"CYSTS."

The "Cholera Cysts" figured in M. Robin's work on "Vegetable Parasites" identified by Hallier.

Dr. Hallier appears to have derived the first idea of cholera cysts from the engravings of the "cholera bodies" of Drs. Swayne, Brittan, and Budd, in the year 1849, as reproduced in M. Robin's work on *Vegetable Parasites.** For, after stating that they are undoubtedly of the same nature, judging from the drawings, of those seen by him, a severe reproof is administered to the French author

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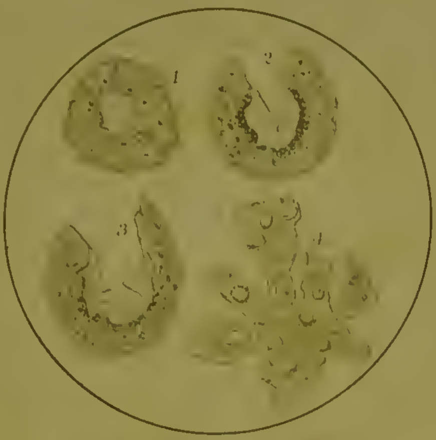
* Histoire Naturelle des Végétaux Parasites. Atlas, Pl. XII., Figs. 4-5.



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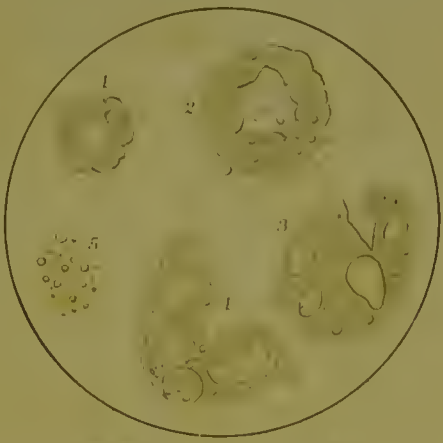

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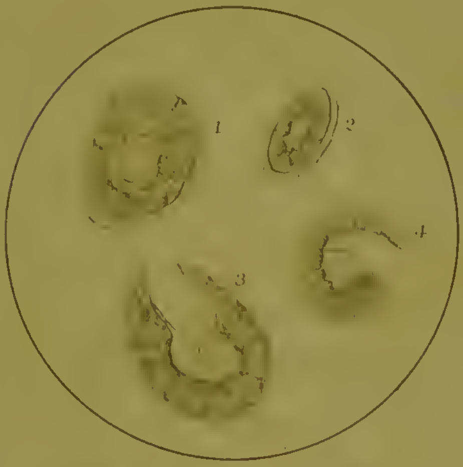

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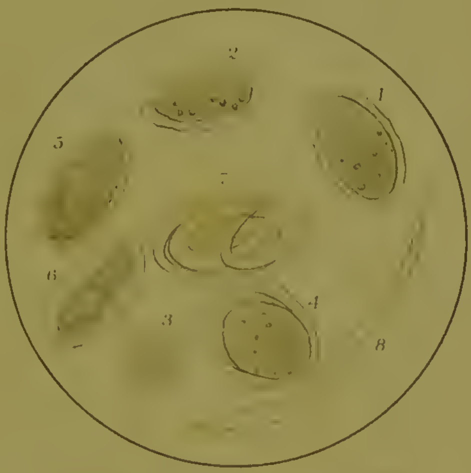

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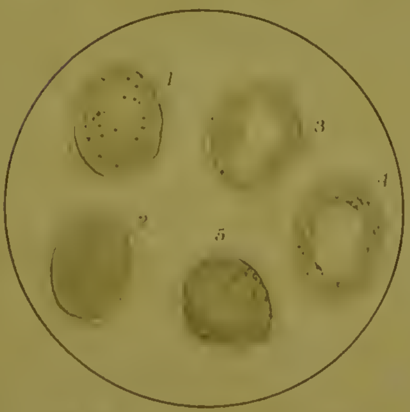

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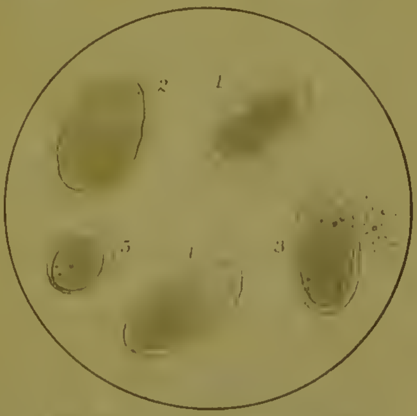

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1 to 5 [illegible] *3.* [illegible]
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FIGS V X "CYSTS" IN CHOLERAIC DISCHARGES

History of these bodies.

Opinion of Mr. Busk as to the nature of these bodies;

of Drs. Baly and Gull.

for the summary way in which he disposed of the "cysts" of the Bristol Doctors. As these "cysts" have been the subject of discussion for more than twenty years, without any definite conclusion as to their real nature having been attained, a few observations concerning them may not be uninteresting. In September 1849, Dr. Brittan published a description of the bodies observed by him, termed "annular bodies," in the *London Medical Gazette;* this term comprising bodies varying considerably in size and appearance—large masses corresponding to Hallier's cyst, and smaller bodies which probably correspond to Hallier's spores. Mr. Brittan did not attempt any cultivation so as to connect the one class with the other, but inferred that they were the same in different stages of development, because he had observed something like a connection between the size with the severity and duration of the disease. The late Professor Quekett, of the Royal College of Surgeons, coincided with him in the belief that they were different stages of the same body, and of a fungoid nature. Mr. Swayne also announced that he had discovered certain cyst-like bodies which were named "cholera-cells," drawings and descriptions of which he published in the *Lancet* about the same time as Mr. Brittan. He also believed that the larger and smaller bodies figured were mere stages in the development of the same thing. Dr. Budd believed that he found similar bodies in the water of tainted districts, and designated them "cholera fungi." These announcements caused considerable excitement at the time, which was somewhat lessened when Mr. Busk announced that the bodies in the sample received by him were a species of uredo (*Uredo segetum*), the bunt of wheat, illustrating his statement by the removal of bodies like the one in question from a loaf of ordinary brown bread. The College of Physicians appointed a Committee of Inquiry, and Drs. Baly and Gull drew up a report, in which the small bodies are said to be either carbonate of lime (probably from the aromatic confection mixture taken), disintegrated blood-cells, or starch particles; the larger ones figured by Dr. Budd to be probably accumulations of starch cells with disintegrated particles of vegetable tissue, and those of Drs. Brittan and Swayne to be some species of bunt, as identified by Mr. Busk. The

Reverend M. J. Berkeley, the greatest authority on fungi we have, on being referred to, declared that the specimens he received were not fungi at all, so that evidently the propounders experienced some difficulty in recognizing their own "bodies," otherwise such microscopical experts as Mr. Berkeley and Mr. Busk would not have been supplied with such entirely different substances.

Of the Reverend M. J. Berkeley.

Here the matter rested until Professor Hallier observed a resemblance between the cysts in the choleraic discharges examined by him, and those figures in M. Robin's book, which figures are here reproduced (Fig. ii), as being the only criterion we possess of what Hallier really means when he speaks of cysts; the only drawing published by him of the mature cyst being that of a ruptured one (i, 1).

Reason for reproducing M. Robin's figures.

In the examination of cholera dejecta which I have made in Calcutta and in the North-Western Provinces, many "cysts" were observed, and these in many cases closely resembled the ones figured in M. Robin's work, but were not of such universal occurrence as the attention they have obtained would have led one to expect; indeed, frequently absent altogether.

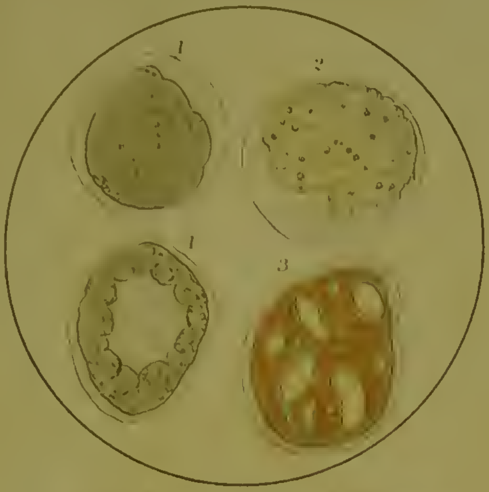
Cyst-like bodies in the choleraic dejecta observed in India.

The "cysts" figured by Drs. Brittan and Swayne (the greater part of which are here reproduced from the drawings accompanying the original articles of these gentlemen) are certainly the kinds most frequently present in evacuations, as the fact that the following observations concerning them were completed before either the original figures or copies of them had been seen, would tend to show.

They may be divided into two classes. The principal figures in Dr. Brittan's drawing will serve as an illustration of one kind (Fig. iii, 1), and the leading figures in Dr. Swayne's of the other (Fig. iv, 1-4). As the two classes are copied in M. Robin's work, and Dr. Hallier does not intimate his belief that they are not of the same nature, it will perhaps be best to allude to the two, so as to leave no stone unturned in the matter. That they vary much in their nature will be manifest from the following observations:—

Two distinctly different kinds of "cysts" figured by Brittan and Swayne.

1. The dejecta of a patient who had been suffering



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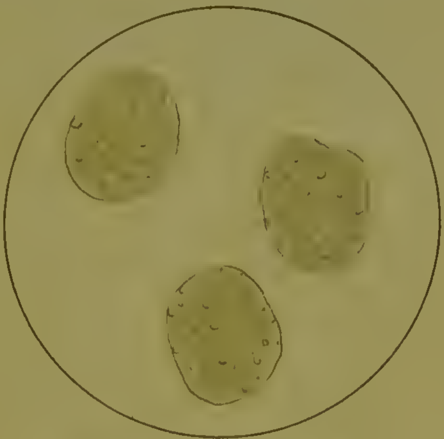

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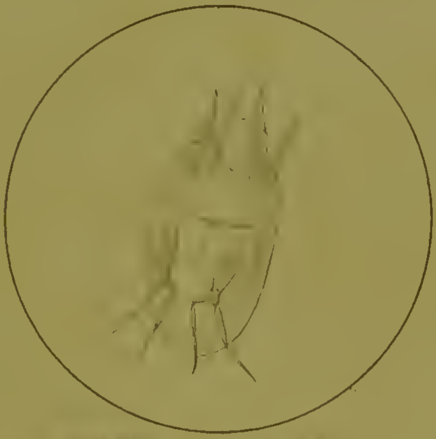

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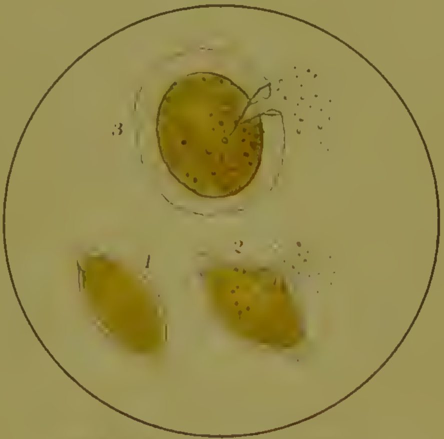

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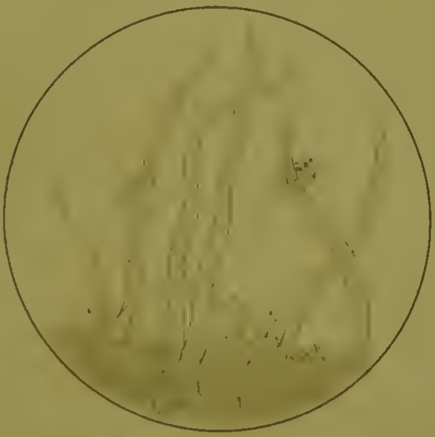

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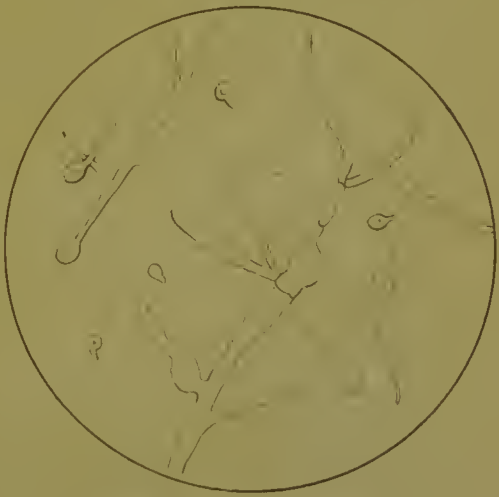

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FIGS XI XVI. OBTAINED IN CHOLERA-STOOL

from cholera about 12 hours, and who died on the second day, presented an enormous quantity of globular masses of a dark-yellow colour, except at the centre, where the colour was much lighter, and the mass was much more transparent than at the side (Fig. v, 1), which is not unlike the ones figured by Brittan. Strong liquor potassæ being added, one of the "cysts" burst, as at 2, and gradually broke up, as at 3, 4. The semi-fibro-gelatinous mass in which the "cysts" were involved was entirely dissolved. Another slide was taken, and two cysts selected, a large one and a smaller one (Fig. vi, 1, 2); strong acetic acid being added, no result followed for some time; pressure was applied, and the large one broke up into air-globules and granular matter (3, 4). Pressure was again applied, and the small one also broke up, as shown at 5. Another case may be quoted as illustrative of this kind of cyst. The evacuation was passed six hours after attack, and two hours before death; it contained numerous cyst-like bodies, some entire, others more or less broken up, and in many cases seemed to contain partitions (Fig. vii). These bodies withstood the action of rectified ether until the fibro-albuminous matter surrounding them had been removed by the application of potash.

Irregular dark-yellow "cysts" corresponding to Brittan's "annular bodies."

Action of liquor potassæ;

of acetic acid;

of ether.

2. In the same evacuation other globular cyst-like bodies were found of a yellowish-green tint, having a more defined outline, and more evenly diffused contents (Fig. viii, 1, 4). These were unaffected by the ether, and remained unaffected by liquor potassæ for three days. These cysts occurred in nearly all the evacuations examined, but their precise nature was for a considerable time unexplained. They were sometimes round, but generally oval, and in some cases formed about a fourth of the entire sediment. This was particularly observed in some dejecta with which I was favoured from the Medical College Hospital, obtained from a native who was admitted with all the symptoms of cholera, but eventually recovered. They were, as in other cases, of a greenish-yellow tinge, with colourless hyaline capsules, for the most part oval (x)—sometimes round, and varying considerably in size, as seen in the figure. One of these cysts was

Well defined, round or oval greenish-yellow cysts unaffected by ether and liquor potassæ, resembling Swayne's cholera-cells.

Action of re-agents repeated;

but no material change observed.

Result of pressure.

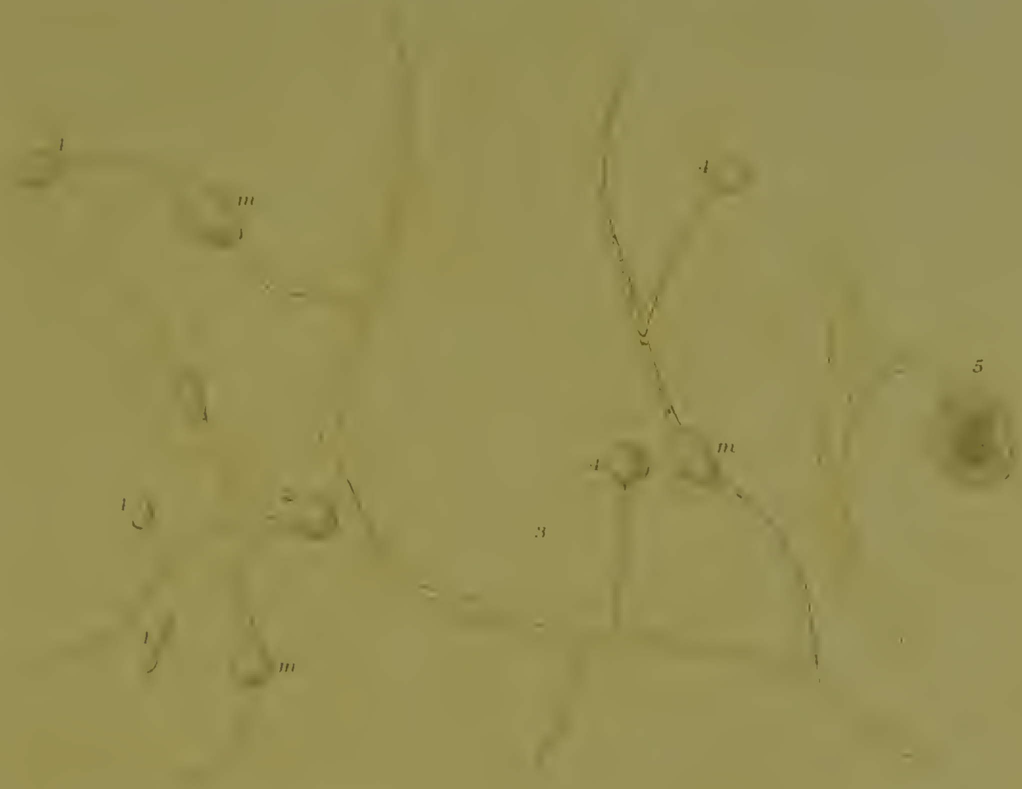
selected for special observation, the one represented at Fig. ix, 1; ether being added, the contents cleared up a little, but nothing further; this was followed by strong liquor potassæ, which caused it to become dotted and streaky, the yellow tinge, however, remaining (2); gradually changing to the appearance depicted at 3, the centre becoming more transparent than the circumference, which still further extended, as at 4. The transparency of the central portion diminished in the course of a few minutes (5), in which condition the object was left under the microscope until the next morning, when it was found to have retained its form, but had acquired a dark colour. Another cyst was selected with a dark-yellow granular centre, and hyaline capsule (Fig. x, 1). Ether was added; scarcely any change; merely clearing up the centre a little. It was then rolled over, and the granular contents spread throughout the entire cell (2). Firm pressure was applied, the eye being kept steadily at the microscope, when suddenly numerous minute molecules escaped (3), and the capsule became partly emptied of its contents (4). Liquor potassæ was added to a portion of the evacuation and allowed to stand all night. The cysts on examination next morning appeared unaffected.

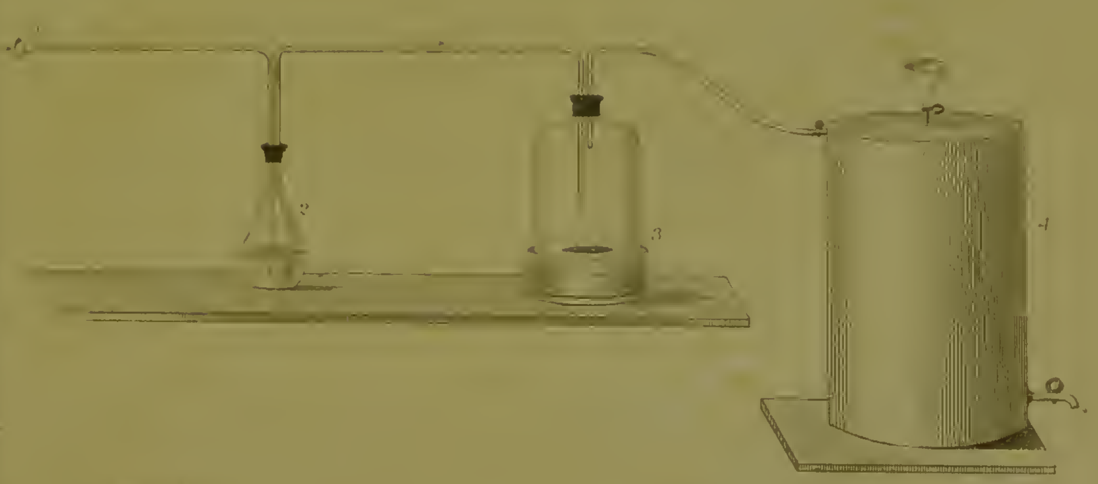
Action of sulphuric acid, iodine, and alcohol.

To another test tube sulphuric acid was added. The cysts after remaining several hours in the acid were not much altered, but presented a globular outline with a hyaline capsule surrounding a greenish-yellow molecular mass (Fig. xi, 1, 2). On rolling them over they became oval, but soon regained the circular form. On the addition of a strong solution of iodine, the contents became dark-brown, and on subsequently adding absolute alcohol, fat-like globules made their appearance, which, by manipulation, could be made to move within the cell; the capsule being unaffected (Fig. xi, 3). Alcohol being added to another cyst without the iodine, the contents assumed a lumpy appearance with a clear space in the centre (Fig. xi, 4).

Embryos of worms associated with the cysts.

Several very small embryos of round-worms having been observed in the evacuation in active motion (Fig. viii, 8), diligent search was made as to their origin, which resulted in the explanation of the nature of the cysts also.





FIGS XVII XVIII

The latter were frequently observed to give evidence of some kind of systematic arrangement of their contents, as shown in the figure (viii, 5, 6), and eventually a cyst was observed to contain something which rolled within it; this, after prolonged watching, was seen to present the exact form and size of the worm-like body just alluded to. It was coiled up on itself within the capsule (Fig. viii, 7), and continually altered its position. This corresponds pretty accurately with the drawing of the ovum of *Ascaris mystax* in Dr. Cobbold's work on Entozoa. It is, I think, pretty much the same as the cholera-cell of Mr. Swayne. In many cases the contents of these ova are also shrunken, occupying a part only of the enclosing membrane, as insisted on by this gentleman as a means of diagnosis. The effects of re-agents also, as above given, correspond very closely with the description given by him.

Contents of the cysts.

3. There is another cyst not very uncommon in choleraic dejecta, having a more delicate, but very resistent capsule (Fig. xii). Its nature may be inferred from the following statement: On two or three occasions, semi-disintegrated *acari* were observed in the stools examined, which had, in all probability, been swallowed with the food, in bread perhaps, and passed through the intestinal canal without being very much broken up, as may be seen from the figure (xiii). It did not, however, occur to me to connect the existence of the thin capsuled cysts with these *acari*, until one day two were seen rapidly depositing their eggs among some fungi under cultivation, which were being microscopically examined. These eggs corresponded precisely with the just described cysts.

Acari, and their ova in cholera stools.

4. Mr. Brittan figures some oblong bodies (Fig. iii, 2), which are not reproduced in M. Robin's plates, but were probably also considered to have some connection with cholera by the author of the article in the *Medical Gazette.* These are exceedingly common, and are accurately drawn in Fig. xiv, where one is seen entire, and another ruptured, together with one of Mr. Swayne's bodies in a ruptured condition; both required the application of considerable pressure before the capsule gave way. The first described elongated body is, I believe, the ovum of another round-worm, the *Tricocephalus* (*dispar ?*). As to the cysts with distinct spore contents, which Hallier has figured (Fig. i) as being a mature

Ova of another round-worm in Brittan's drawing.

condition of the cysts comparable to the drawings in Robin's work, I have not met with any which were unmistakably the same in fresh dejecta, but have developed them repeatedly; the particulars will be given further on. Other cyst-like bodies are occasionally found, but as they do not in any way correspond to those of the author of the theory under consideration, a description of them is reserved for another occasion; the principal ones, however, are those already described, namely, (1) *compound cysts*, consisting of fragments of various tissues and fat surrounded by a semi-organized fibro-albuminous layer, and (2) *ova* of various kinds, none of which are peculiar to cholera.

Summary of the principal cyst-like bodies observed in cholera.

As, however, the ultimate elements of other cysts than these might exist in the dejecta, every known method was resorted to for the purpose of developing them, a few illustrations of which I give in a condensed form.

Illustration I:—

Small portions of the dejecta which contained such numbers of the cysts, alluded to in page 7 and represented at Fig. v, were placed in three perfectly clean watch-glasses with the following substances:—

I.—Cholera evacuation 3 drachms, and 2 drops of acetic acid, so as to neutralize it.

II.—Cholera evacuation 3 drachms, phosphate of ammonia 3 grains, grape-sugar 3 grains.

III.—Distilled water 3 drachms, phosphate of ammonia 3 grains, grape-sugar 3 grains.

To receive these, a small wire stand had been placed in a shallow dish containing a strong solution of permanganate of potash, and the stand and watch-glasses covered in by a bell-glass (carefully cleaned, and subsequently rinsed with alcohol) which stood in the fluid. This was set aside in an average temperature of 82° *Fahr.*

On the third day small white specks were seen on the surface of No. I, which had returned to its alkaline condition, one of which was picked out as rapidly as possible from beneath the bell-glass and placed on the stage of the microscope. It consisted of an aggregation of minute molecules held together by a slimy substance, from which filaments of fungi escaped (Fig. xv). Thus matters stood until the fifth day, when from No. II being picked out, presented numerous spores

Progress of the cultivation.

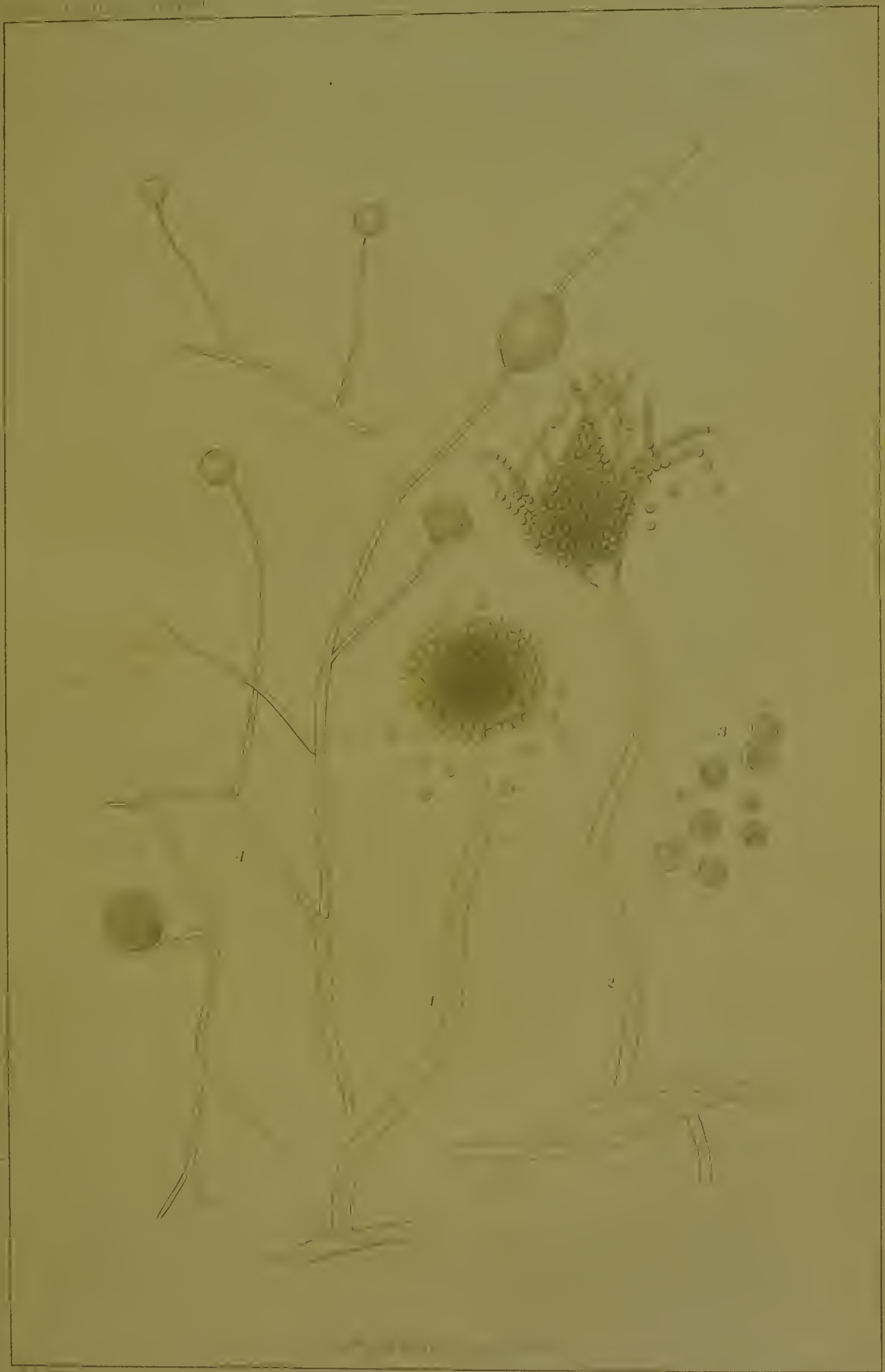


FIG XIX FUNGI. DEVELOPED IN CHOLERA STOOL

(Fig. xvii, 1), many of them germinating very actively (2), and the filaments here and there were swollen out into macroconidia (3, m), some of these dilatations being transparent, others granular; frequently the filaments were seen to terminate in a bulb (4), and in one case a filament was tipped by a cyst in which the contents were granular and had contracted from the capsules (5). Precisely similar filaments and dilatations were found in No. I, but a distinct cyst (or sporangium) could not be seen. This condition lasted until the seventh day, when the mycelium gradually degenerated, and a crop of aspergillus appeared on all three, of various colours, but principally of the dark varieties.

One cyst developed.

After the seventh day aspergillus appeared in all.

Illustration II:—

A portion of the fluid contents of the small intestine from a patient who had died within six hours of attack was carefully transferred to a vial, and allowed to settle for an hour. In the meantime a "growing" solution was made, consisting of grape-sugar 3 grains, phosphate of ammonia 10 grains, glycerine 1 drachm, and distilled water 1 ounce. A drop of this was placed on three glass slides; to these were added:—

Growing solution.

No. I.—A minute quantity of the upper layer of intestinal contents.

No. II.—A minute quantity of the sediment chiefly.

No. III.—A minute quantity of diabetic urine containing "yeast cells."

These were placed as before under a bell-glass placed in Condy's fluid; on the third day specks appeared on the preparation in each slide, which proved to be due to spores and mycelium (Fig. xvi), the three slides presenting similar appearances under the microscope. On the fourth day No. I presented an excellent forest of penicillium, and No. II a similar crop of aspergillus, of the black and purple coloured variety, while No. III produced both penicillium and aspergillus. These were systematically examined for eight days, no other fungus making its appearance. The aspergillus crop in No. II presented tufts of different colours; specks were observed in the other two preparations; a speck of yellow and brownish-purple being the most abundant.

Spores germinating in each preparation on the third day.

Only penicillium and aspergillus appeared.

Isolating apparatus described.

To experiments conducted in this manner, there is the serious objection that each time the preparation is examined, no matter how carefully, the possibility exists of foreign matter getting into the preparation. With the intention of obviating this source of fallacy as much as possible, an aspirator was employed to supply the preparation with purified air, at least as pure as passing it through concentrated sulphuric acid will allow. By referring to the accompanying sketch, it will be readily seen how this was effected (Fig. xviii). A small funnel (1) with a pledget of clean cotton wool inserted into its neck was attached to a piece of bent glass-tubing; this tubing passed through a perforation in the cork of a flask (2) containing concentrated sulphuric acid; from the neck of this flask another piece of glass-tubing emerged which connected it with a perforated bell-glass, standing in a shallow dish containing Condy's fluid; (3) another piece of tubing connected this with the aspirator (4) filled with water. All the connections were carefully luted, so that the only air which could have got at the preparation on the stand within the bell-glass (of course *minus* the air which previously existed therein) must have passed through the sulphuric acid.

Illustration III:—

A perfectly fresh choleraic evacuation having been obtained two hours before death (in a rapidly fatal case lasting only seven hours), three watch-glasses were placed in the isolating apparatus with the following ingredients:—

Substances placed in the isolating apparatus

No. I.—A slice of the interior of a plantain weighing quarter of an ounce was scooped out, and six drops of the sediment from the evacuation was placed in the little cavity thus made.

No. II.—A few drops of the evacuation-sediment only.

No. III.—A slice of the same plantain as in No. I.

The apparatus had been made as clean as possible previous to this, rinsed out with spirit immediately before depositing these glasses on the stand beneath the bell-glass, and the greatest care taken to avoid foreign matter getting at the preparations before placing them there. The air within was renewed morning and evening; the weather was warm the whole time, the average day temperature of the room being about 90° *Fahr.*

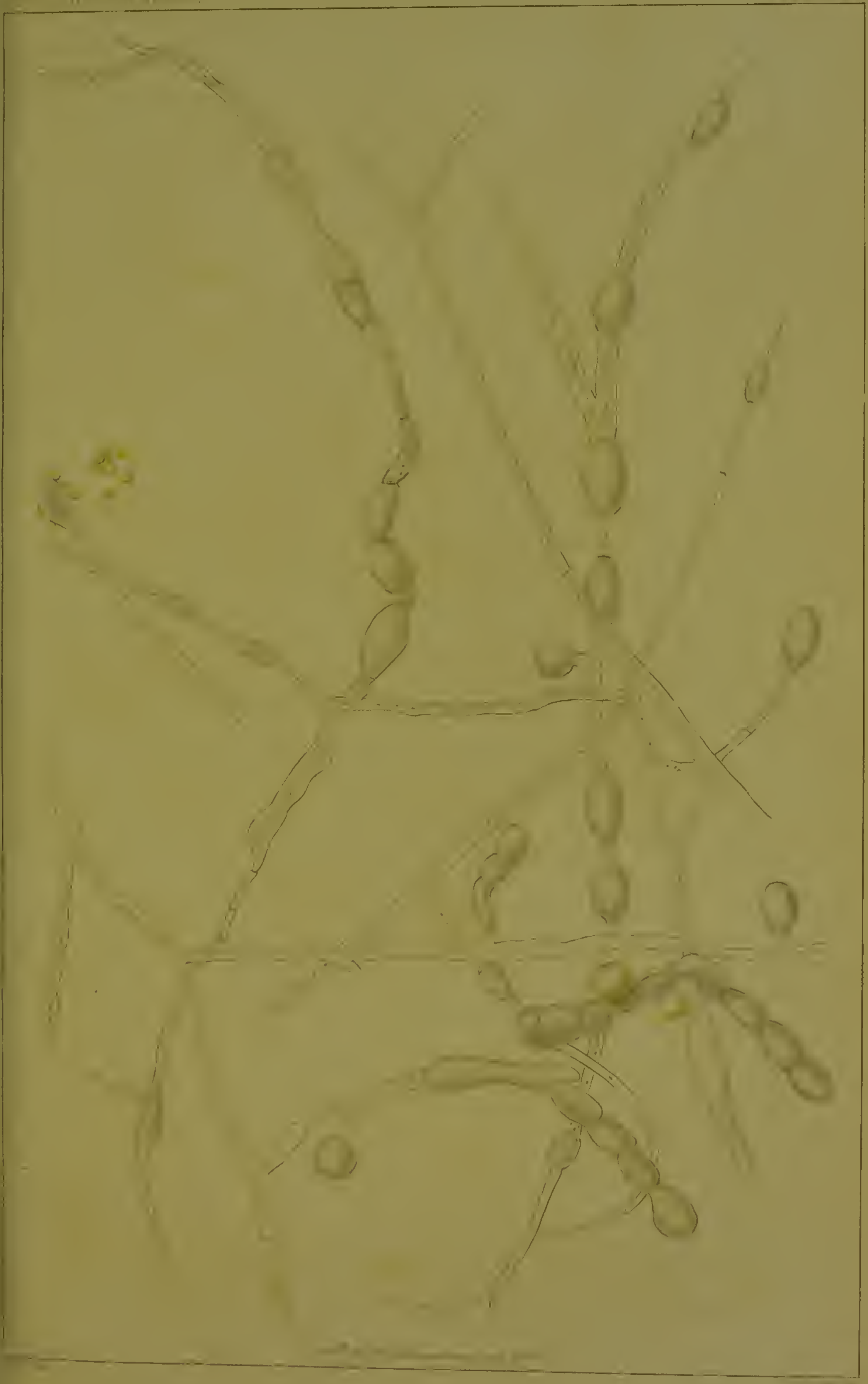


FIG XX. MYCELIUM WITH MACROCONIDIA BUT WITHOUT SPORANGIA
DEVELOPED IN CHOLERAIC DISCHARGE

allowed to remain in the apparatus for three weeks.

On the fourth day a mould was seen to appear on the two slices of fruit, quite as marked on the clean plantain as on the other, but no change was visible in the watch-glass containing the evacuation only. This condition lasted a fortnight, the crop of fungus gradually increasing in the two former, and no change could be observed in the latter. During the third week the fungus not having made any progress, and the liquid in the watch-glass No. II becoming rather less, from evaporation, the apparatus was opened on the twenty-fourth day, and the result carefully examined forthwith.

Condition of the fruit on removal.

The two pieces of fruit were covered with a thick coating of a black and yellow coloured fungus, both colours appearing in the two preparations; the yellow prevailing in the tainted slice, and the black on the other; the difference being merely in the proportion, for tufts of each colour appeared here and there over the surface. These were found under the microscope to be *aspergillus* (Fig. xix, 1) and *penicillium* (xix, 2). Precisely the same fungus and the same species grew on glycerine, on starch-paste, and on pieces of dirty cork in various parts of the room. In the other watch-glass, however, containing the evacuation only, a very different appearance was observed. The preparation had become partly dry, and presented a filmy appearance. On placing the watch-glass on the stage of the microscope, a great quantity of spherical bodies were seen with granular contents, the average size being about that of a white blood-corpuscle, but the size varied considerably, among which long delicate mycelical filaments ramified (Fig. xix, 3); from this network thin fertile threads arose, tipped in most instances with exceedingly delicate vesicles (xix, 4), which appearance at first was taken for the dew-drop aspect so common to mycelium; others were seen of a much larger size. On watching them closely, all the bodies were seen to roll round and round, like a *volvox*. Elongated (spore-like) bodies were distinctly visible within each delicate capsule, unless very small (xix, 5), and seemed to move irrespective of the capsule (or sporangium): of this, however, I am not certain. They appeared white by reflected light, and yellowish-green by transmitted light. The

The fungus developed in the watch-glass with evacuation only.

Description of the delicate cysts.

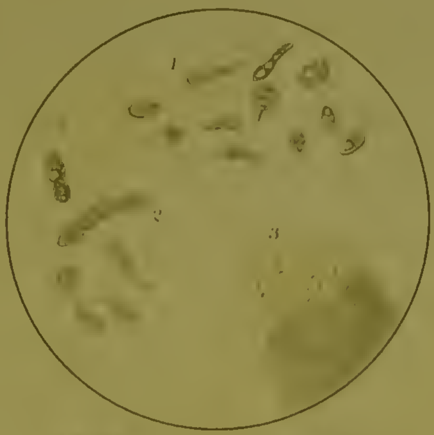
movement appeared to me to be due to currents of air in the room, each little sphere twirling round rapidly in one direction for ten or twenty turns, then as rapidly twirling in the opposite way. The course of the spinning vesicle was not always horizontal, but varied until it was nearly vertical to the filament on which it was perched, but never quite vertical. It seemed analogous to the spinning of a plate or ball nicely pivoted on a juggler's stick, which may be seen to revolve in every direction but the vertical, the analogy being complete, except that the organic connection between the sporangium and the stalk rendered reverse turns necessary. On touching this with water, the capsule appeared to become instantaneously dissolved, no trace being left: the spores had fallen down, and the filament looked perfectly bare. Some parts of the mycelium were dilated into saccules (or macroconidia) (Fig. xix, 6), but no evidence of spore contents was distinguishable.

Illustration IV:—

Cultivation of a cholera stool obtained during an epidemic of the disease.

Being desirous of ascertaining whether from the rice-water stools in epidemic cholera I could produce capsules more unmistakably like those figured by Professor Hallier than I had succeeded in doing from discharges obtained in an endemic locality, such as Calcutta is, a sample was brought from Lucknow, carefully secured in a clean vial, which was obtained during my visit to the North-Western Provinces during the epidemic of cholera which occurred there in September last. A drachm of the sediment was poured into a perfectly clean watch-glass, and placed on the stage in the isolating apparatus in the manner described in the last illustration. In the course of a week a film was seen to have formed, which continued to increase in density for another week, but no trace of any mould could be observed in it through the bell-glass. At the end of three weeks the preparation was taken out and microscopically examined, but no cysts had formed, as in the former preparation treated in exactly the same way, but there was a great quantity of mycelium, in the meshes of which numerous circular bodies were embedded (Fig. xx); the latter seemed to be the result of segmentation of the former, judging from the similarity

No cysts were developed, but a quantity of segmented mycelium.



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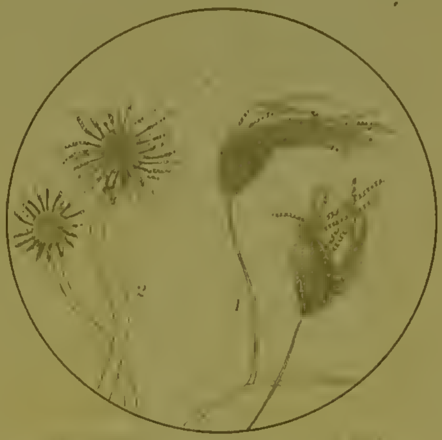

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FIGS: XXI XXIII DEVELOPED IN ORDINARY EXCRETA

between the free cells and the imperfectly detached segments of mycelium. The watch-glass was replaced in the apparatus for a fortnight, but no change took place.

From these illustrations it will be seen that whereas cysts, distinctly resembling those described by Professor Hallier, may, by *cultivation*, be observed to develop in choleraic discharges, yet they are by no means constantly obtainable, for out of more than a hundred cultivations, made with the express object of developing these cysts, only three times was I able to produce any fungi bearing such tokens of fructification.

Is it possible to develop fungi in other than cholera dejections bearing fruit resembling the "cholera cyst?" The answer must be "Yes," as the following experiment will show:—

Illustration V:—

Cultivations of ordinary healthy stool, one isolated and the other exposed.

About half an ounce of fæces, obtained from a perfectly healthy person, was placed on a small glass plate, and carefully transferred into the bell-glass of the isolating apparatus in connection with the aspirator, as already described, the greatest possible care having been taken to prevent foreign matter coming into contact with it before depositing it on the stage in the apparatus. A small portion of the same substance was placed on a glass slide, without any special precautionary measures being taken to prevent access of foreign matter, so as to be able to examine it from day to day for comparison with the preparation in the bell-glass, which it was not intended to disturb. On the second day a few small white spots were observed on both preparations, one of which was picked out with a needle from the non-isolated mass, and placed on the stage of the microscope. It consisted entirely of minute molecules, round and elongated (Fig. xxi, 1), embedded in a white shining substance (2), in connection with which were circular and oval cells of a greenish tint (3); frequently two or more were seen strung together (4); clear spaces were seen in them all nearly.

Progress of exposed preparation.

On the fourth day the mass in the apparatus was completely coated by this white humus, except that some of the earlier observed spots had acquired a yellowish-brown colour. The exposed slide presented a somewhat similar appearance. The cells had become nearly everywhere strung together, and long filaments of *oidium lactis* (Fig. xxiii), corresponding exactly to the figures given by Thomé of the cholera fungus discovered by him, to which rather a long name was given at the time, *viz.*, "*Cylindrotænium Choleræ Asiaticæ.*"

The "cholera fungus" of Thomé.

This condition lasted till the sixth day, when a crop of a white mould was perceptible in the isolated preparation, and a plentiful crop of *penicillium* and *aspergillus* appeared on the other cultivation (Fig. xxii). This slide having become rather dry, a few drops of distilled water were after this occasionally added. On the eighth day long delicate filaments were seen growing out of the white humus-looking substance in the apparatus, and on the tenth day other filaments were observed, which seemed to be tipped with various coloured heads, apparently of the same kind as on the other slide, those of a bluish and yellowish-brown tint prevailing; but by the eighteenth day the long delicate filaments had grown over them, the whole surface of the preparation presenting a woolly appearance. After this no further change could be seen to take place in either cultivation, and on the twenty-first day of the experiment the bell-glass was opened, and the glass plate placed on the stage of the microscope. Precisely the same species of *aspergillus* and *penicillium* were found as existed in the non-isolated cultivations, with the addition that great numbers of the filaments forming the white flocculent tuft bore at their terminations cysts or sporangia filled with distinct spores (Fig. xxiv, 1-4), which, I think, correspond exactly to the cysts figured by Professor Hallier of the immature cholera-cysts, whose drawing has already been given and may be compared with this.*

Condition of the preparation under bell-glass after first week.

The apparatus opened on the twenty-first day.

Cysts obtained exactly like the "cholera-cysts" figured by Hallier.

Aspergillus tufts were present in great numbers: nearly all of them had fallen off from their filaments among the mycelium; a few, however, were perfect, consequently easily recognised. Some

A germinating aspergillus tuft simulating a "cholera cyst" in the same condition.

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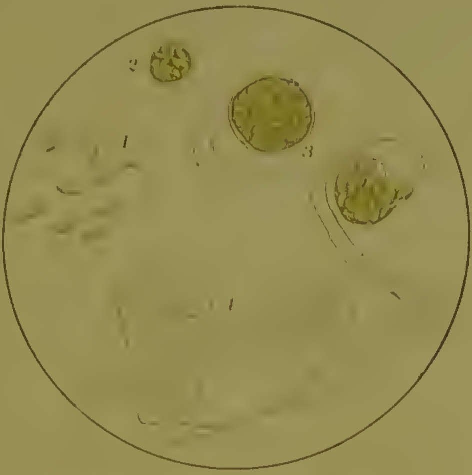
* I have obtained excellent examples of this fungus *(Mucor)* on the intestinal mucous membrane of the pig also, whilst subjecting strips of the intestine to continuous observation.



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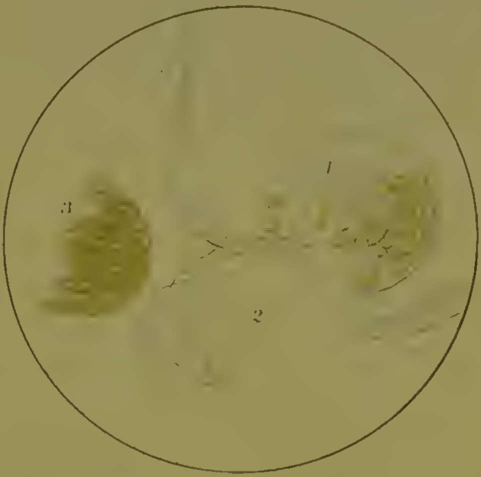

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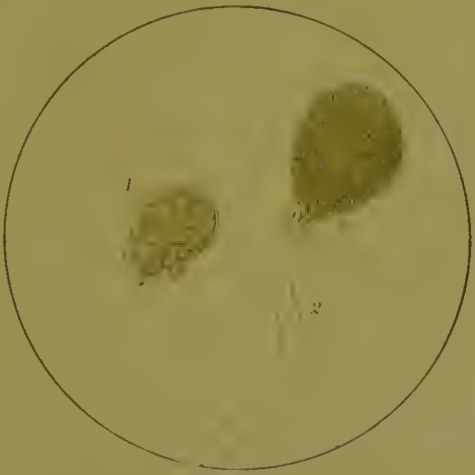

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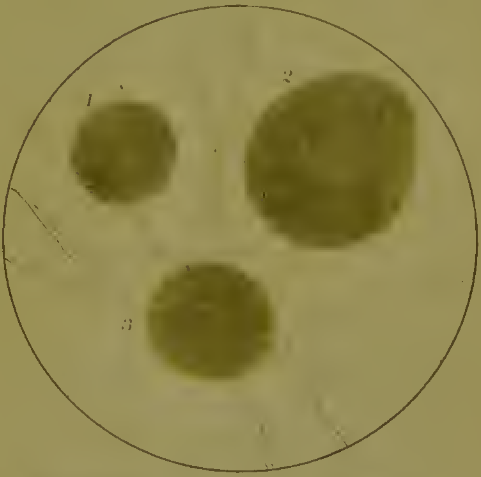

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CYSTS &c. SIMULATING HALLIER'S CHOLERA FUNGUS.

DEVELOPED IN ORDINARY EVACUATION

of these fallen masses were germinating (Fig. xxvii, 2), and presented, as nearly as anything possibly could, the appearance of the mass of spores figured by Professor Hallier as a "cholera-cyst" in process of germination (Fig. i, 11).

As the preparation was dry, a few of the cysts were transferred to another slide and water added, upon which many of the capsules of the sporangia gradually ruptured, and the spores escaped (Fig. xxv), a bare columella and the ruin of the capsule alone remaining.

As it was not advisable to expose the preparation during the experiment, the various stages in the development of these cysts were not followed, in order to ascertain which some spores and cysts were sown on the juice of various fruits, boiled and unboiled, and on pieces of cheese. They rapidly germinated, and in those preparations which were sown in cells on the slide without a covering glass, produced precisely similar cysts to those sown; when, however, covering glasses were used, the fructification was not so perfect. For example, a glass slide was taken, and two semi-circles of asphalt varnish were brushed on it, one being rather larger than the other, so that the ends of one half-circle might overlap the other, but not so closely as not to permit the entrance and exit of air, as may be learnt from the Figure (xxix). When nearly dry, a minute quantity of growing fluid, consisting of a solution of grape-sugar and phosphate of ammonia, was placed in the centre, upon which a few spores were sown, a thin covering glass being placed over it, which adhered to the semi-dried varnish. The slide was placed under a bell-glass, kept damp by being lined with some moist blotting-paper, at an average temperature of 90° *Fahr.*

The cysts and spores sown on a slide, and the development described step by step.

In the course of six hours a clear oil-like spot appeared in the spores, and on the second day they were germinating rapidly (Fig. xxviii, 1). On the third day the field was crowded with mycelial filaments (Fig. xxviii, 2), and on the seventh day a filament which had crept beyond the *droplet* of fluid into the free space between it and the varnish bore a distinct sporangium (Fig. xxviii, 3). Separate spores, however, were not distinguishable in this cyst.

Deductions.

These illustrations will, I think, be sufficient evidence to show—(1) that the cholera-cysts figured by Professor Hallier are not always obtainable from choleraic discharges, (2) not confined to cholera, (3) nor even to diseased conditions of the intestine, but (4) may be cultivated from the stool of perfectly healthy persons.

Inoculability of rice plants.

The experiments instituted to test the observation as to the inoculability of rice plants have as yet not been satisfactory, consequently no conclusions have been arrived at on the matter.

SECTION II.—"SPORES."

Corpuscles simulating the drawing given of "spores" by Hallier.

It is by no means so easy to explain what the yellowish more or less oval hyaline bodies are which Professor Hallier calls "spores" (*vide* Fig. i, 3); such bodies are exceedingly common in choleraic discharges, and I believe are very different in their nature; but whether any of them are "spores" will, I think, be satisfactorily explained in the sequel. The objects I have met with in cholera discharges more or less resembling these bodies may be arranged into four classes:—

(**1**).—*Globules of a fatty nature ;*
(**2**).—*Altered blood-cells ;*
(**3**).—*Corpuscles embedded in the tenacious substance composing the "flakes ;" and,*
(**4**).—*Globular conditions of certain infusoria.*

The frequent difficulty of recognizing globules of a fatty nature from other globules.

1. Persons accustomed to microscopic work must have found that to distinguish fat or oil globules from other bodies very different in their nature, is not always so easy a matter as is commonly stated in text books on the subject. It has frequently occurred during this investigation that, in spite of the addition of heat, absolute alcohol, rectified ether, potash, iodine, and other re-agents, not overlooking the prolonged application of carmine, I have failed in distinguishing with certainty fat globules from pellets of slimy substances endowed with life, when both were known to be present. Indeed, I have frequently mixed fat with gum water and other substances for the purpose of testing the value of the re-agents which had been applied to bodies under examination, and have found that, in a great number of instances, the results are fallacious; either the globules remain unaltered, or both

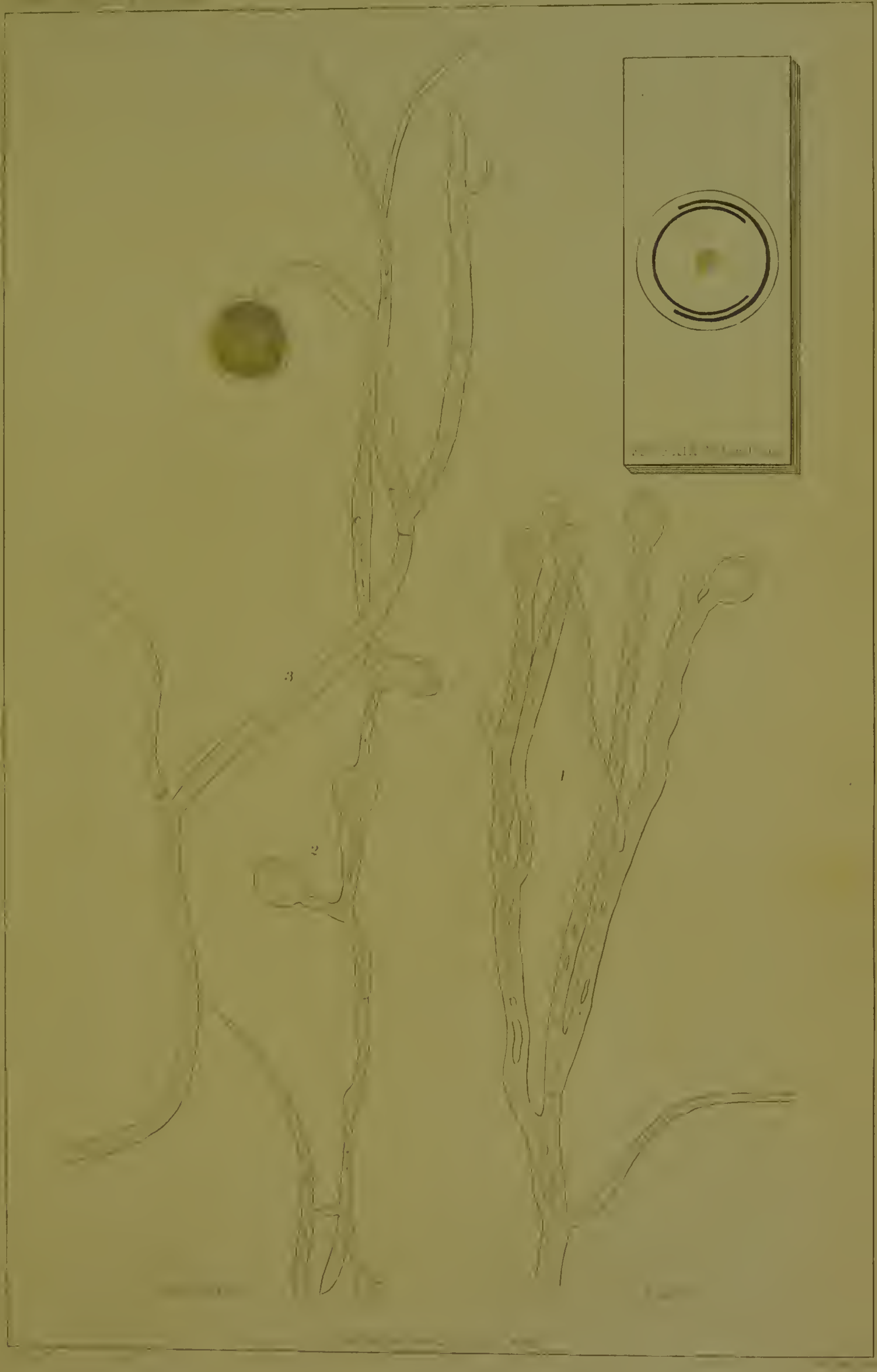


FIG XXVIII FUNGUS VERY LIKE HALLIER'S "CHOLERA FUNGUS"
DEVELOPED IN THE GROWING SLIDE XXIX FROM ORDINARY STOOL

kinds are destroyed, or they are acted upon indiscriminately. A fair sample of this difficulty is carefully delineated at Figure xxx, representing objects very like delicate "cysts" and "spores," which being watched for eight hours remained unaltered, resisting pressure, &c., but broke down in twenty-four hours into unmistakable globules of oil.

Having experienced very great difficulty in this matter, I propose giving one more example of a condition which is a particularly prominent feature in the early stools of a cholera patient; indeed, for a long time I was unable to persuade myself that it was not a condition of some low form of life, especially when the globules were highly coloured, or when the homogeneous contents of the pellicle shifted its position.

A sailor was admitted into hospital with all the symptoms of cholera, and, at the time this evacuation was obtained, suffered from severe cramps. The stool was examined three minutes after being voided, was found to be alkaline and of a muddy colour. The sediment consisted almost entirely of greenish-yellow corpuscles, varying considerably in size, the larger ones being flattened out under the covering glass (Fig. xxxi); many of these having the contents contracted, the contour of a delicate, filmy capsule being evident at the spot where shrinking seemed to have taken place (Fig. xxxii). They were generally spherical (1), but many were oval (2), and a few were seen presenting several hyaline projections whilst rolling in the fluid on the slide (3). In some cases they retained their form and appearance for a long time, but the greater number lasted only for a few hours. They were frequently observed to vanish suddenly like a distended blood-cell, leaving only a ring behind (Fig. xxxiii, 1), previous to which, in a few instances, a slightly granular appearance was presented (2), and the ring was often seen particularly granular (3, 4, 5), as if all the contained granules had adhered to it. The globule in the centre of the figure, with the contents separated from its enclosing pellicle (6), was watched for a long time, but no alteration in its appearance occurred. Other similar bodies were watched continuously for three hours with the same result, save that they gradually became excessively transparent, visible only by careful adjustment of the mirror. In the course of four or five hours the entire field presented the appearance delineated in the figure last alluded to. At Figure xxxiv a regular colony is seen

Various forms assumed by globules of a fatty nature.

of these globules surrounding a crystal. They also disappeared in the course of a few hours.

Effect of re-agents.

Rectified ether caused the pellicle to present a minute granular appearance, and those which had the contents puckered became symmetrical. Boiling in ether seemed to thicken the pellicle. A portion of this was set aside until the next day, and was found to have retained its condition, whereas the globules in the evacuation set aside in the vial had disappeared.

Absolute alcohol subsequently added to the boiled portion seemed rather to diminish their number. In some cases one globule was observed to "melt" into the other, so as to form one globule; otherwise no change was observable.

Solution of chloride of zinc and iodine,—some became shrunken and irregular, others continued spherical, but with a finely granular pellicle.

Solution of iodine only caused several of them to become very transparent—scarcely visible, were it not for the slight tint communicated to them.

Liquor potassæ causes them to lose their yellow colour; they become perfectly transparent, except that a few molecules which existed within are brought to view. A few of the globules withstand the re-agent for some time.

Acetic acid seemed to coagulate the pellicle, as it became finely granular: very much the same appearance as followed the addition of alcohol.

Dilute sulphuric acid caused the contents to contract, but the colour was retained, or it became slightly brown.

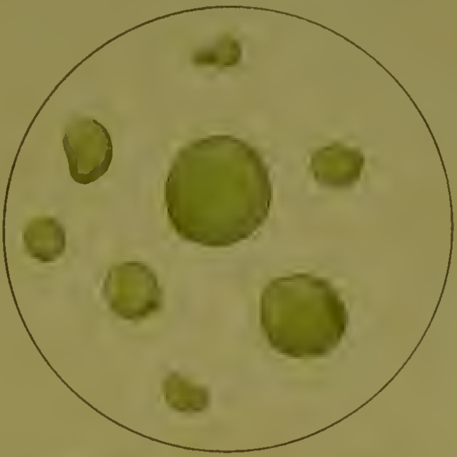
Dilute nitric and hydrochloric acids acted in the same way.

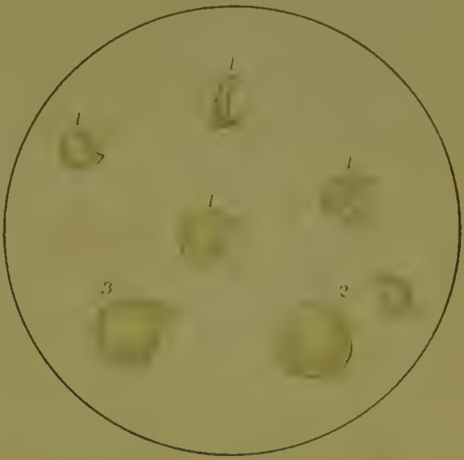
Their artificial production.

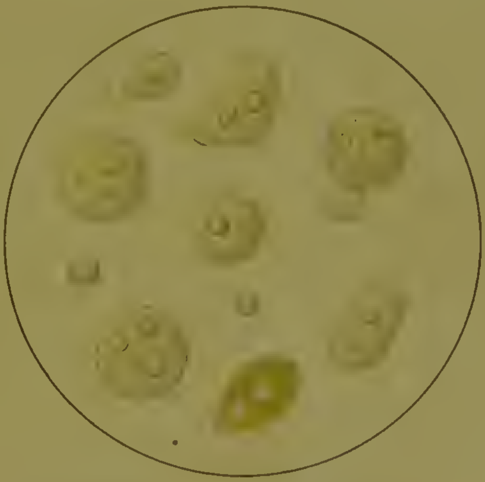
I have made many attempts artificially to produce globules of this kind, the nearest approach being a mixture of melted butter, albumen, and gum water well shaken together, and at the time of examination adding a little thick syrup so as to cause the puckering to take place between the pellicle and the contained fat. The action of re-agents, however, on this pellicle was slightly different to the foregoing.

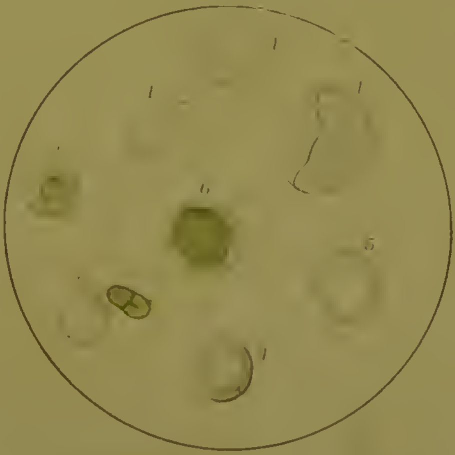
Distension of spores by fluid.

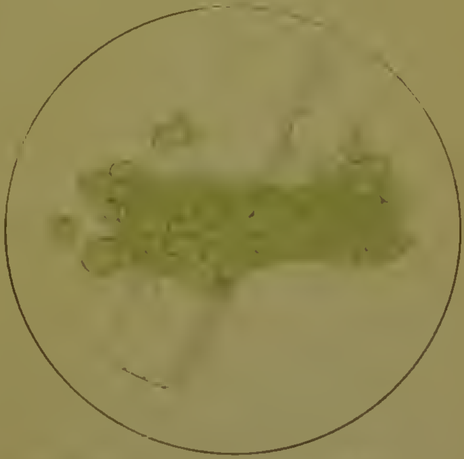
Spores immersed in fluids of varying density become greatly altered in their appearance; frequently the outer layer becomes so attenuated, and perhaps stained, that it is a matter of great difficulty to state positively that the cell pellicle surrounding the protoplasm of a spore











FIGS XXX XXXIV GLOBULES OF A FATTY NATURE
SIMULATING CYSTS, SPORES, &c.

differs from the clearly defined outline of a globule of oil, in spite of a knowledge of the action of re-agents, and of the varying powers of refraction which liquids manifest. Hence it is not impossible, nor inexcusable, that Professor Hallier in some instances might have been deceived by these appearances, especially as it is evident from the conclusions he draws concerning the importance of some of the "cysts" in M. Robin's plate (which are undoubtedly fat), that he had not made prolonged microscopic examinations of ordinary excreta: the Professor, however, had more spore-like objects to deal with than fat, such as the ones described in the next and following paragraphs.

Altered blood-corpuscles.

2. Almost invariably circular cells are observed in choleraic dejections of a greenish-yellow or brownish tint; contents generally homogeneous, and the capsules very delicate.

The exact microscopic appearance of one of these.

The microscopic appearance of one of these capsules is here represented at different distances from the object-glass, the size selected being about the average (Fig. xxxv). The appearance of the capsule a little before the focus is attained is shown at 1, a clear spot shading off into a dark ring. On bringing the object-glass nearer to it, the defined outline of a spherical body is seen with slight opacity in the centre (2); and on attaining the exact focus, a greenish-yellow perfectly hyaline sphere is brought to view (3). On going beyond this, a dark spot is seen in the centre, gradually shading off towards the periphery (4); when the light is shut off almost entirely, a slightly irregular space is seen presenting a very slight pink tint (5); this particular cell was constantly watched for three hours, when suddenly it became transparent, and required most careful illumination and focusing to make it visible, a delicate ring of a slightly diminished diameter being all that remained (6).

One or more vesicle-like protrusions.

These, however are not always spherical; frequently a very filmy tongue-like projection is observed (Fig. xxxvi, 1), sometimes more than one (2); it is projected exceedingly slowly, and then retracted amœba-like. After a time this action ceases, the projected vesicle-like tongue is either permanently retracted, or is left out rolling about with the corpuscle in the fluid (3). These are doubtless distended blood-cells, a great number of which may exist without yielding the slightest trace of colour to a rice-water evacuation.

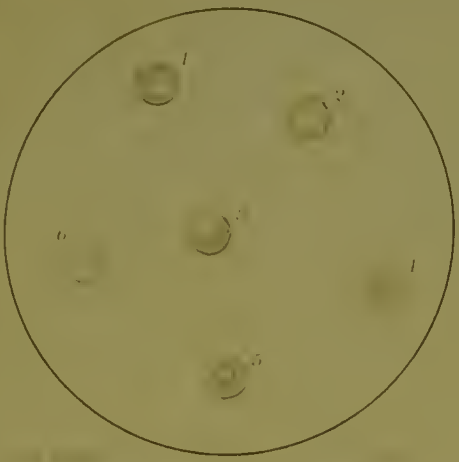
Precisely similar appearances observed in a case of "*Chylous urine.*"

Whilst following the changes taking place in these particular corpuscles in various fluids, I had opportunities of making an examination of the urine of a patient in the General Hospital under the care of Dr. Lyons, who had been suffering from the condition known as "*Chylous urine*" for about a month, together with pain in the right testicle, and great emaciation, in spite of good food and a good appetite. As the colour so closely resembled many rice-water stools, I carefully examined it, and was repaid in a way I had not anticipated. It was albuminous to the extent of about one-fourth of its bulk, slightly acid, with a specific gravity of 1·015.; ether caused a separation into two layers, a clear urine-like fluid containing oil molecules, and a white homogeneous mass consisting of minutely molecular *débris*. Before the addition of re-agents the fluid under the microscope so closely resembled the condition of a cholera stool just described, as not to be distinguishable from it; yellowish-green cells, some hyaline, some granular, some protruding a tongue-like prominence, and others with the contained plasma puckered in various ways (Fig. xxxvii). A few of the larger corpuscles were seen to shift themselves (like an amœba) a distance fully their own diameter, the shape altering at the same time. At first I doubted that they really were blood-cells, as the extent of variation in size was considerable, as shown by reference to the figure, which is carefully drawn to scale. The fluid very quickly gelatinised in the test tube; indeed it frequently does so in the patient's bladder, giving rise to stoppages during micturition. I have not seen cholera discharges spontaneously gelatinise, although such a condition is said to occur. A portion of the coagulated mass (which when stirred closely resembled a lump of moist gluten) was teased on a slide with needles and examined. It consisted of fibrillæ studded with blood; granular cells, scarcely differing from those seen in cholera discharge flakes, except, perhaps, in being more universally granular. They seemed to present more of the character of pus-cells.

Gelatinisation of the urine.

Appearance of coagulum.

In the midst of this fibro-albuminous matter several *embryos of a Round-worm* were discovered every time the urine



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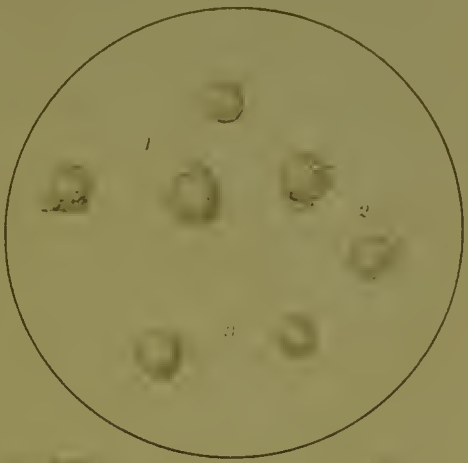

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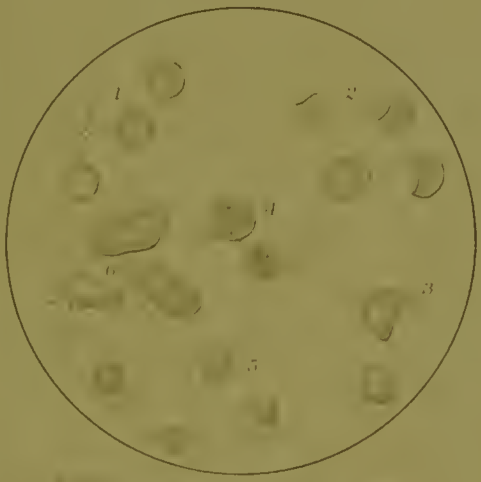

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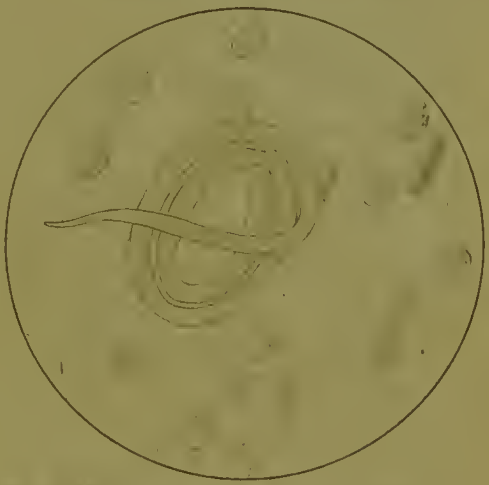

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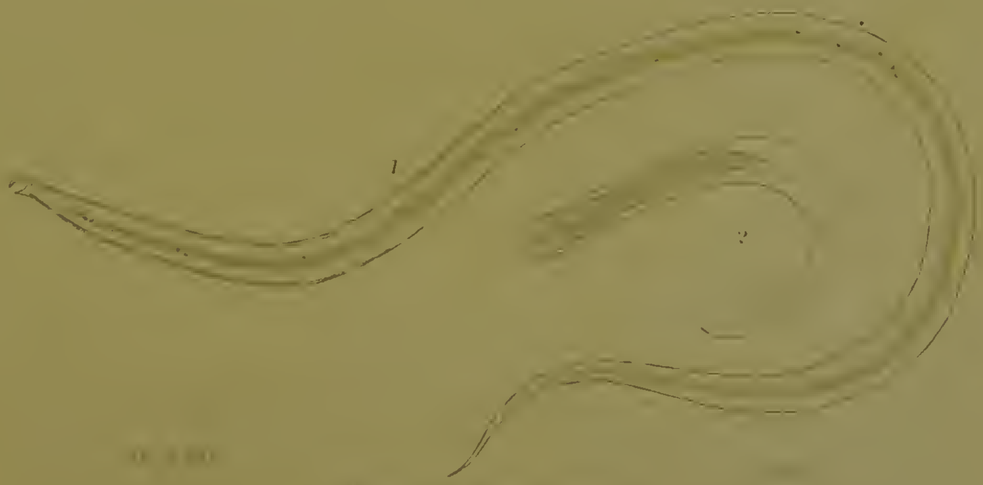

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FIGS XXXV XXXIX

was examined, one of which is seen coiled up in the drawing (Fig. xxxviii). A careful sketch of a larger one, after the addition of acetic acid, is given at Figure xxxix. In the course of a few minutes, when the sketch was nearly completed, a *caudal-bursa* became visible under the influence of the acid, and is delineated at No. 2.

The embryos of a round-worm detected.

When first seen, I thought they were some detached filaments of a fungus, judging from the hyaline, structureless appearance presented; after a time, however, a few of them were observed to move very slowly, when all doubt as to their nature was at an end. It will not be surprising that the existence of these was not suspected, when we consider that fully two hundred of the larger size figured could pass abreast through a very small pin-hole, an orifice not exceeding the fiftieth of an inch in diameter, as may be verified by a simple calculation.

Approximation of diameter.

Perhaps this fact may help to throw some light on a very obscure disease, of which little is known beyond the symptoms, although frequently met with in some parts of the world; and, indeed, may perhaps account for its localisation to such places as the West Coast of Africa, where I am told it is by no means a rare malady.

As the mature worm still retains a hold on its victim, being perhaps safely lodged in the kidney, and not having seen an embryo of this kind before, nor yet a drawing, I must leave to a more experienced helminthologist to decide to what species of nematode it belongs.*

3. In examination of this class of corpuscle, namely, those intimately associated with the well-known flakes in cholera dejections, it is of the greatest importance that the evacuation should be a recent one, because its character may be entirely changed in the course of an hour or two. Sometimes, however, the change is not so rapid, depending on the chemical nature of the fluid, especially on the extent of its alkalinity—cholera stools being almost invariably alkaline. The method adopted in these examinations is to pour the discharge into a conical

The corpuscles associated with the "flakes" change in appearance very quickly.

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[*] While this report was passing through the press, the "*chylous*" condition which this urine had presented for more than two months gradually disappeared, and so did all traces of albumen, and of the embryo-worms.

vessel, set it aside for a short time, and, when the sediment is seen to have been deposited, a pipette is introduced in order to transfer a portion of it to the slide. Frequently the sediment is seen to be of a very slimy nature, requiring some tact in bringing it into the pipette.

Illustration I:—

Case exemplifying these corpuscles in an early condition.

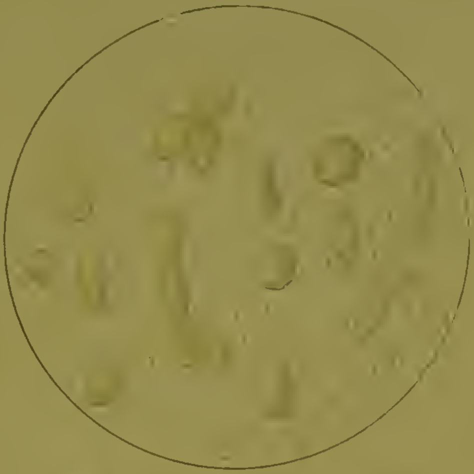
The evacuation was from a man suffering for eight hours from a severe form of cholera, who died on the second day. It was of a pale straw colour, with a muco-flocculent deposit. In the upper liquid portion nothing special was visible, but on examination of the sediment, it was found to consist of flakes of a gelatinous semi-fibrous texture, studded with globules, circular and oval, with a pale yellow tint, and of a homogeneous nature, a very correct representation of which is given at Figure xl. In some of these bodies a clear space is observed, but nothing further could be made of their nature.

Iodine stained some of a brownish-red, and others of a deep yellow.

Effect of re-agents.

Liquor potassæ seemed to make the corpuscles more distinct at first, and to isolate the contained granules and molecules, giving the contents a distinctly dotted appearance. The fibrillated substance became slightly granular, then it gradually faded, and so did the corpuscles, which in the course of half an hour entirely disappeared, except here and there a little cluster of molecules, five or six, with a clear space in the centre, all trace of the fibrillated texture having disappeared.

Acetic acid increases the stringy appearance at first, making each little fibril appear dotted, like a very fine bead of granules, or minute molecules; eventually the fibrillated appearance is obliterated altogether, a diffused, finely granular substance being universal. The corpuscles maintain a sharply defined outline; the continuity of the outline, however, seems frequently somewhat broken in one or two places, as if the circle were formed of two or three short vibriones imperfectly united at their ends. The next day the sediment was still slimy, and could not be taken up by means of a delicately pointed pipette. It still consisted of a



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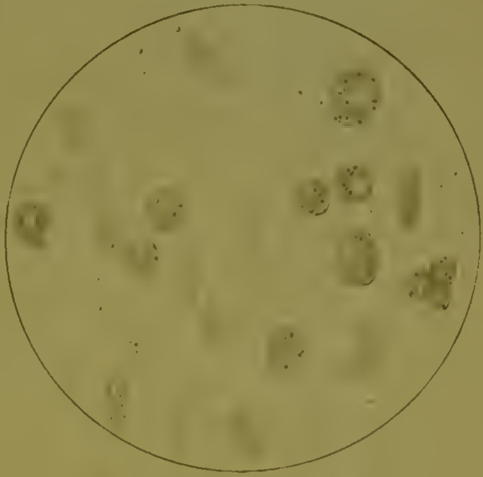

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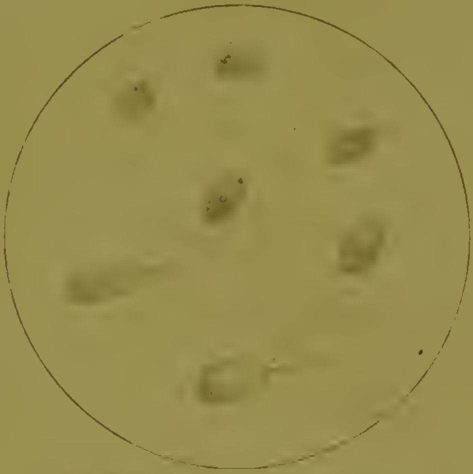

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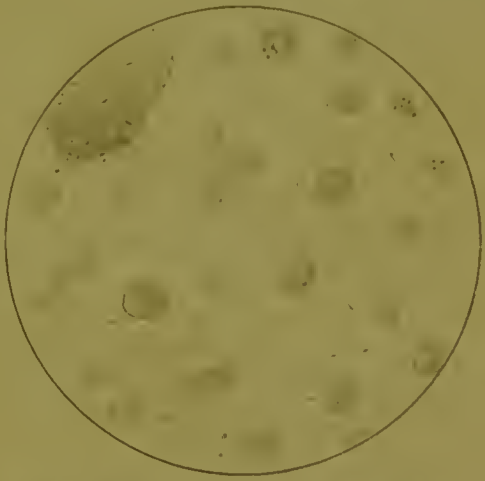

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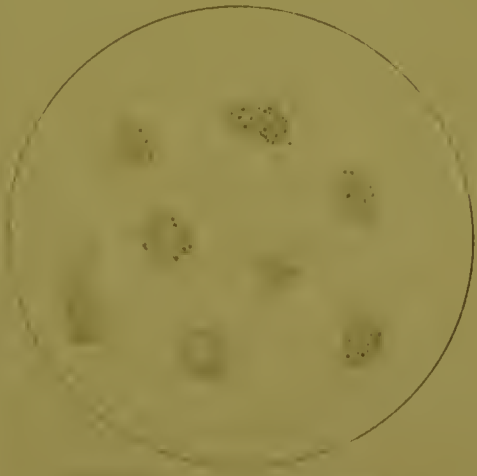

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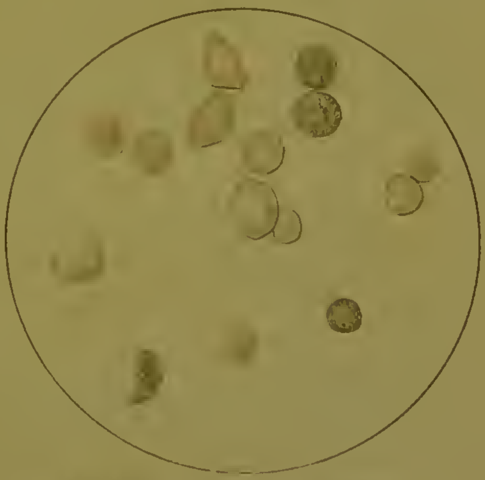

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FIGS XL XLV FLOCCULI &c IN CHOLERA STOOL

The appearance on the second and succeeding days.

streaky, semi-membranaceous substance, but the imbedded cells had either become transparent, or presented a granular or minutely molecular appearance (Fig. xli), with no distinct cell wall. Solution of chloride of gold picked them out very distinctly.

On the third day the flakes had lost their membranaceous character altogether, but many of the granular corpuscles remained. On the fourth day a few animalculæ were seen, which enormously increased by the fifth (Fig. xlii). On three occasions only have I observed the appearance of *this* protozoon in choleraic discharges.

Several evacuations from the same patient were subsequently examined; the flakes, however, did not present the fat globule-like appearance again, but molecular, as shown in the previous Figure (xli).

Illustration II:—

Case in which the corpuscles were granular when the evacuation was voided, and exhibited amœboid movements.

Another case, the third liquid stool, presented a yellow colour, about one-sixth of which was composed of a whitish flocculent sediment, presenting precisely the same microscopical character as the second stage of the last described; a semi-membranaceous substance, dotted with irregularly defined cells (Fig. xliii), very like what is seen in exudations effused in catarrh. On very careful watching they are seen to protrude excessively delicate processes of an amœboid character (Fig. xliv), just as the white blood-corpuscles do.

Effect of re-agents.

Liquor potassæ caused the membranaceous appearance to vanish after a time, reducing the cells to an aggregation of granular or molecular particles. *Ether* does not destroy them, nor does *acetic acid*, but it seemed to make manifest a delicate cell wall; and *iodine* superadded enhanced this appearance, in many cases causing the contents to collect at one part of the cell (Fig. xlv).

The membranaceous appearance had disappeared in the fluid on the fourth day, but the granular cells remained visible for nearly a week.

Illustration III:—

Case in which hyaline and granular bodies are seen together.

The fifth evacuation of a patient suffering from the cold stage of cholera was examined half an hour after it was passed. It was colourless, with a few shreddy flocculi floating in it. It was slightly alkaline. The flakes presented the same membranaceous appearance as in the foregoing example (Fig. xliii), with numerous corpuscles, more or less intimately held in the meshes of this texture, a great number, however, being dispersed in the fluid; some were oil-like and some granular, examples of both kinds being spherical and oval, and the gradations from the merest particle of slimy or oily matter to the complete corpuscle were so fine, that it was impossible to point out any salient distinguishing character about them. When free, the hyaline and granular corpuscles were more or less round, but when contained in the meshes of this fibrillated texture, were generally elongated, as shown in the drawing (Fig. xlvi).

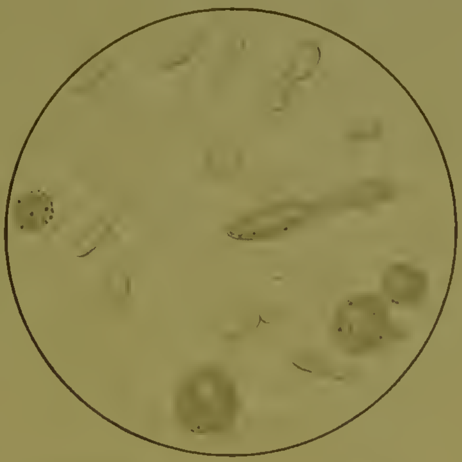
Effect of iodine.

Iodine solution being added to the slide, it was observed that whereas some of them were coloured brownish-red, the greater portion became merely stained by the ordinary tint of the iodine (Fig. xlvii); all, however, in the course of the day becoming granular, but the distinction of brown-red and mere yellow remained.

In the course of an hour other slides were prepared, but the microscopic appearance had become totally different. The oil-like bodies, of whatever shape, had become granular, and the field presented exactly the same appearance as presented in Figures xli and xliii, while the addition of reagents produced the same results. On the fourth day all traces of corpuscles had passed away, merely broken down molecular matter remaining.

The "still" circular condition of animalculæ.

4. Intermixed with the corpuscles already described are others to which I wish to allude with the greatest caution. Frequently a globule has been observed for some time, and finally disposed of as being merely an oil one, when suddenly it is seen to protrude a portion of its substance; retract it, and while so doing another protrusion



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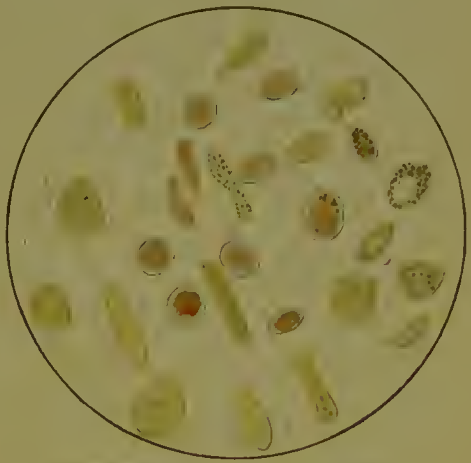

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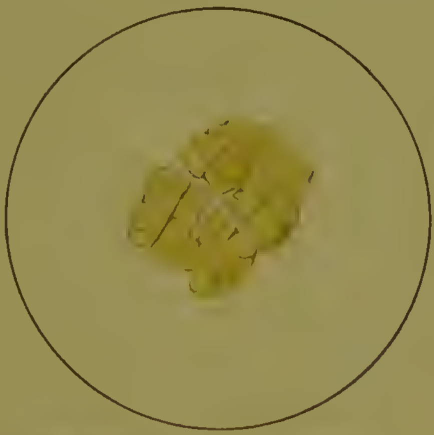

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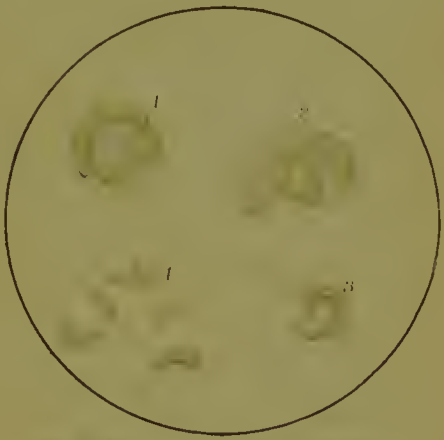

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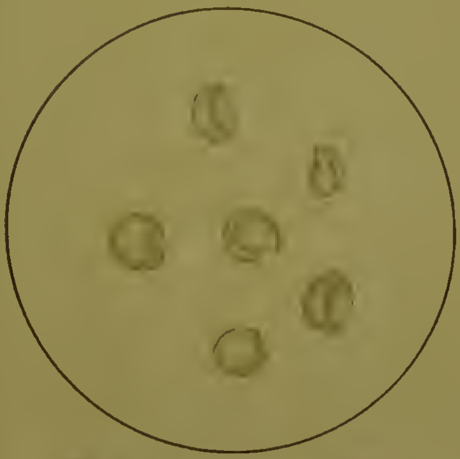

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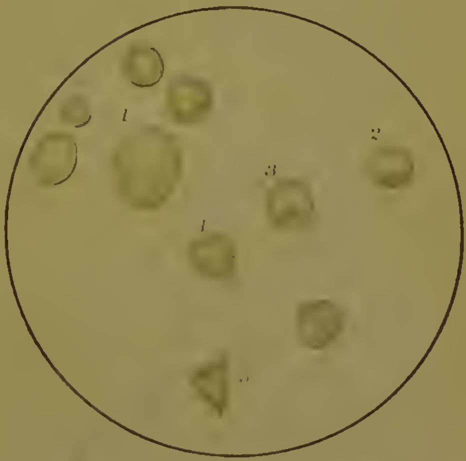

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FIGS XLVI LI VARIOUS OBJECTS IN CHOLERA STOOL

becomes visible at some other portion of the little mass, and then, perhaps, it will shift its position, exactly after the manner of an amœba.

These are frequently hyaline in a fresh stool, but generally granular; no trace of nucleus or contractile vesicle can be observed; sometimes they are very numerous, but when there are other corpuscles in the field which act in a somewhat similar manner, it is impossible to say to which class they belong, unless, indeed, they move across the field like an ordinary amœba, and not merely content themselves with protruding portions of their substance into the surrounding fluid, as was stated the corpuscles in the last described kind did. I am not in a position to state that these are the "still" and amœboid conditions of more than one kind of animalculæ; probably they are, but that they are so of one kind, I think I may state pretty definitely; and, as they are sometimes distinguishable in the still globular condition for a considerable time, they really may have been the bodies seen by Professor Hallier, and mistaken by him for swollen spores; most frequently, however, they are of short duration.

Are frequently hyaline, but generally granular.

Possibility of their having been mistaken for swollen spores.

The cause of this variableness I am not in a position to state.

These bodies were noticed very early in the course of the inquiry, and every particular concerning them noted; but I have to confess that not a few links are wanting in the "life history" of these animalculæ, which the following illustrations will but too plainly demonstrate.

Illustration I:—

A pale, straw-coloured, perfectly liquid stool, in which the sediment was very scanty, was obtained from a patient in the cold stage of cholera. The dejections being passed involuntarily, numerous little heaps of *sarcinæ* were present (Fig. xlviii), as indeed exist to a greater or less extent in nearly all the cholera evacuations examined, with numerous masses of a granular or jelly-like substance, in which yellow translucent lumps are imbedded, probably of a fatty nature (Fig. xlix, 1); together with masses of a somewhat similar outline observed to alter in form very slowly, as at 2. In some cases a pellicle becomes evident, when the contained

Presence of sarcinæ almost universal in cholera stools.

jelly-like protoplasm contracts, as at 3, the various forms assumed by which are represented at Figure l, with a great number of more or less spherical bodies very like oil globules (Figure li); some are seen to be flattened out (1), others protruding a vesicle exceedingly slowly; the body at No. 2 becoming in the course of five minutes to the condition delineated at 3, 4, 5; whilst great numbers of a minute animalcule were seen actively moving among them all; sometimes one flagellum is seen a posterior one, at others an anterior one also, both being retractile at will, and another may be darted forth out of any portion of its body. No organized structure can be seen, neither mouth nor eye spot, nor any trace of contractile vesicle, merely a spindle-shaped speck of jelly enclosed in a delicate elastic sac, endowed with the power of rapidly altering its shape and position (Fig. lii). So capable are they of adapting themselves to circumstances as to be able to insinuate themselves with the fluid through the meshes of fine blotting-paper. All these were present to a greater or less extent for a week.

Alterations in the form of the spherical bodies very gradual.

Presence of exceedingly active animalculæ;

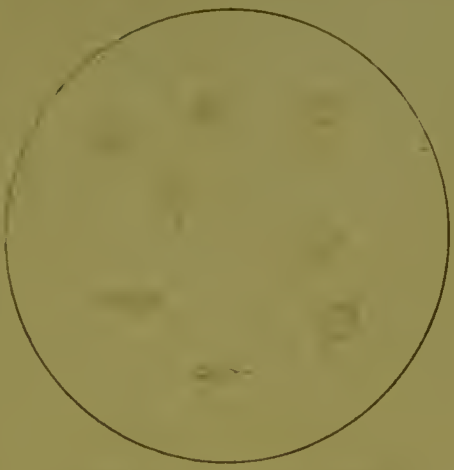
structureless; perpetually changing in form.

A drop of the fluid was placed on a hermetically sealed slide, and the little bodies remained active until the fourth day, when they gradually ceased to present any kind of motion, but settled down into irregular little masses of jelly-like appearance, to which condition also the corpuscular bodies had been reduced (Fig. liii). On several occasions, however, the animalculæ were seen to become more than usually active for a short time, before ceasing altogether: to push out processes in all directions, and as quickly taking them in again, finally settling down as shapeless little pellets. Some of the various forms assumed by *one* of these at this stage are sketched in Figure liv.

Transition from the active to the "still" condition.

Illustration II:—

A condition precisely similar to the foregoing was observed in the evacuation of another man a few hours before death, as well as in the contents of the large and small intestine at the *post-mortem* examination. The action of re-agents is much the same as on any other hyaline protein globule.



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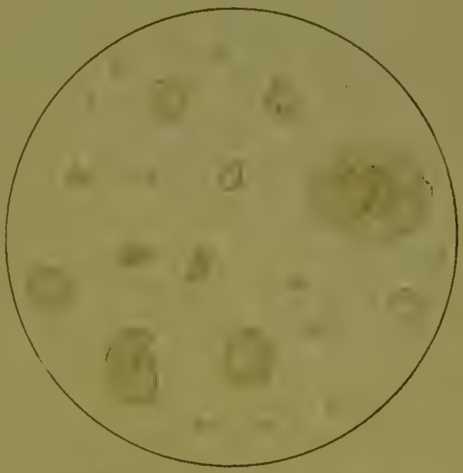

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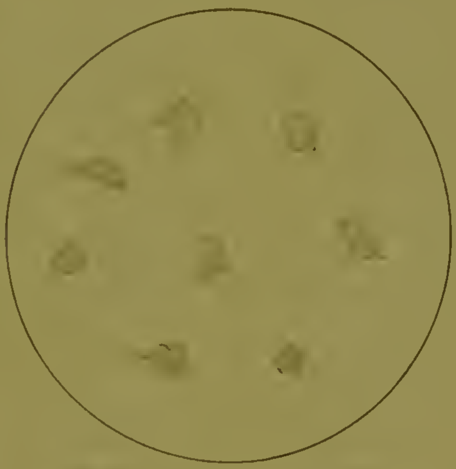

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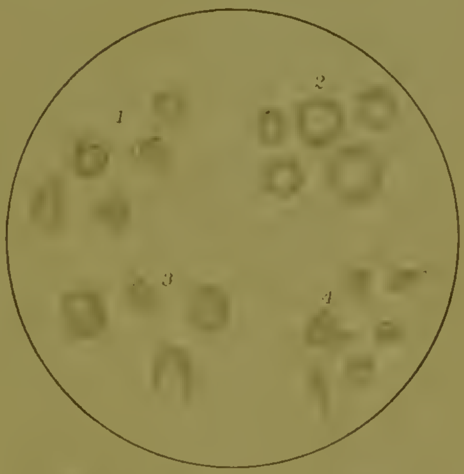

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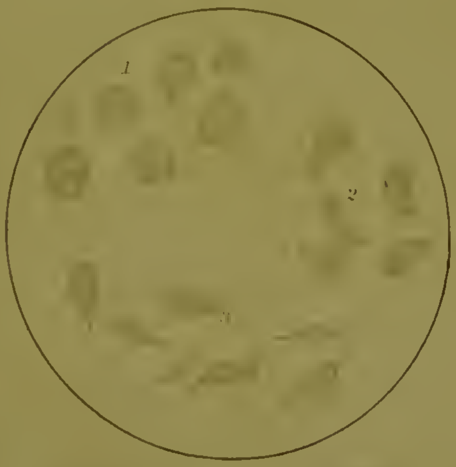

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FIGS. LII LVII STAGES IN THE EXISTENCE OF THE ANIMALCULE
AND THE REV [illegible] BERKELEYS GROWING CELL

Action of re-agents.

They remained unaffected by strong *acetic acid* for ten minutes; gradually, however, the contents contracted more or less regularly, thus allowing of a delicate capsule being brought to view (Fig. lv, 1). After a time the contents vanished, merely a finely granular ring being left (2); *absolute alcohol* made the contents appear granular, as at 3, whilst some appeared but little affected; *ether* subsequently being added caused them to shrink considerably, but did not dissolve them (4).

Iodine stains them a brownish-red and makes them appear somewhat granular (5).

In order to test whether some of them might not be "spores," a series of observations was commenced, some of which have already been described in the chapter on "Cysts" (page 11).

Mr. Berkeley's growing-cell.

A growing-cell was prepared on the Revd. Mr. Berkeley's plan, by drawing a ring of varnish on the glass slide, allowing it to become nearly dry, cleansing it, as well as the covering glass, thoroughly with spirit and distilled water. A *droplet* of the evacuation was then transferred to the centre of the cell (Fig. lvi), care having been taken that no part of the sides was touched by the fluid when the covering glass was applied.

It was afterwards hermetically sealed, sufficient air being already enclosed to allow at least of germination. The limited area of the preparation enabled the geography of various objects to be easily remembered, and tended very materially to precise observation.

On the second day the corpuscular bodies appeared to be more granular or less like oil globules, frequently with one or more indistinctly visible vacuolæ (Fig. lvii, 1); many are elongated and presenting very slight movements (lvii, 2). A few animalculæ were still present; germinating spores were also visible.

Transition of the "still" to the active condition on the third day.

On the third day the circular and oval bodies had almost entirely disappeared, but on approaching the margin of the fluid immense numbers of the animalculæ, to the extent of half the field of the microscope, were seen moving about with great rapidity and perpetually altering their form, a clear space being observed in some of them (Fig. lvii, 3).

On the fourth day the activity of the little animalculæ had diminished, many were gradually re-assuming the circular condition. Thinking that this was an indication for a fresh supply of air, the varnish was scratched away from a small portion of the side with a needle, watching the effect under the microscope while doing so. They did not appear to be particularly affected by this proceeding, for in the course of an hour they had all become circular, and almost motionless; many attempts were made to get at a more complete life history than this, but hitherto without success.

Return to the "still" condition.

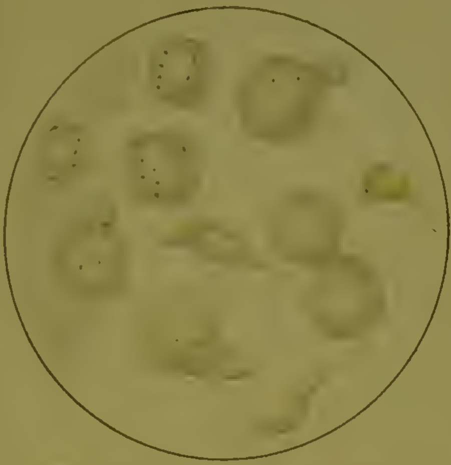
The duration of the corpuscles and of the active animalculæ is very variable, sometimes easily recognized in stools which have been kept for a month; on other occasions disappear in a few hours. They have frequently been seen after having been thoroughly dried to re-assume active movements on the addition of fluid; but exposure to the sun at a temperature of 120° *Fahr.* stops all movements, no matter in what fluid they are placed, becoming sometimes completely disintegrated, but they will re-appear in such a fluid after a time under favourable circumstances—probably new ones being developed. These bodies are not confined to any particular stage of cholera, as the following will prove.

After becoming dried may be revived by the addition of fluid.

Illustration III:—

The dejection of a person, shortly after the first symptoms of cholera set in, was obtained for examination. It was about the third liquid stool, of a pale yellow colour, slightly alkaline to test-paper, with the average amount of sediment. This consisted almost entirely of the bodies sketched in Figures lviii to lx. *First,* a number of large granular cells, very delicate filmy spheres, rolling about under the covering glass (Fig. lviii) frequently, as if undergoing the process of division; *secondly,* corpuscles of the same granular appearance, but generally somewhat smaller, from which filmy vesicle-like projections were seen to proceed very, very slowly, and as slowly retracted, followed by a similar protrusion from another portion of its substance (Fig. lix, 1), or two or more may be seen at

Circular corpuscles presenting amœboid alterations of shape, associated with blood-cells and animalculæ.



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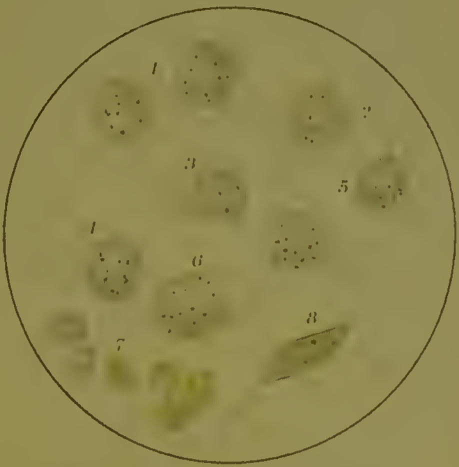

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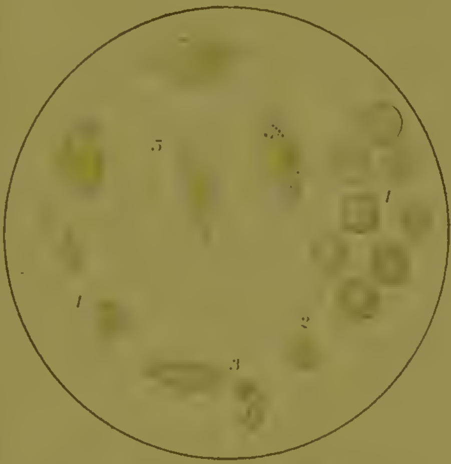

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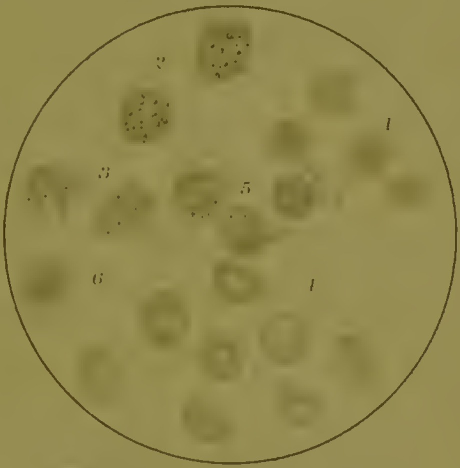

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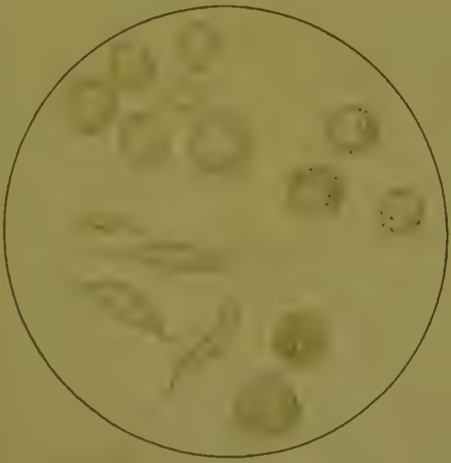

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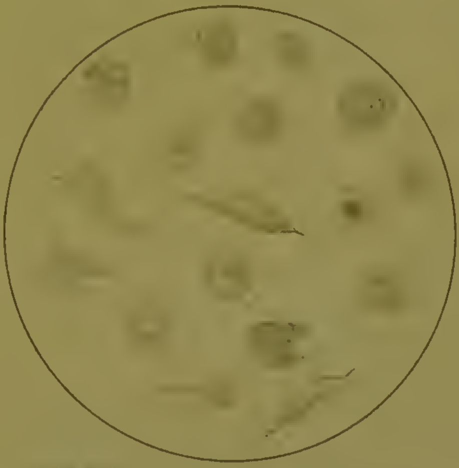

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FIGS LVIII LX EXAMINATION OF CHOLERA STOOL
LXI HEALTHY BLOOD IN DITTO
LXII LXIII ORDINARY STOOL

the same time (2). The granular and minutely molecular matter did not enter into these saccules, and I am not certain whether an inner or an outer wall exists, but sometimes it seemed very like as if the outer gave way for a filmy inner lining to come forth (3), at other times it seemed quite the reverse (4). After a short time the projections in many cases appeared no longer to be retracted, and were seen to curve upon the cell as the evaporating fluid bore it along (5). Some, however, are seen to be of larger size (6). *Thirdly*, blood-cells which have assumed very peculiar outlines, the result of *diosmosis* (7); and *fourthly*, innumerable animalculæ, of the kind already alluded to, exhibiting great activity amongst the various cells in the field (8).

Animalculæ attaching themselves to blood-cells.

The patient died on the next day, but a stool was examined a few minutes before death; it was highly coloured with blood and contained a great number of animalculæ (Fig. lx). Some of these were tugging at the blood-cells and altering their form, distinctly pulling the pellicle or cell envelope away from the enclosed plasma. No. 1 was altered to 2, 3, and 4 in the manner described and shown in the figure. The animalculæ presented an unusual appearance; either a large clear space existed in most of them, of the same size as the blood-cells, or one or two blood-cells had become engulphed in their homogeneous substance. In some cases they were distinctly seen to be merely adherent, the little creature rushing along as if it had a burden.

The next day the blood-cells had become granular, but the animalculæ were as plentiful as ever, and continued so for a fortnight, everything else having broken down.

Are any of these corpuscular bodies peculiar to cholera?

Having now given a brief account of these few classes of corpuscular bodies, and shown that none of them were seen to germinate like the spores of fungi, the question naturally arises—Are any of them peculiar to cholera?

The *first* and *second* classes of corpuscles.

The *first* class, namely, those of a fatty nature, need not be considered, for no one will suppose them to be peculiar to the disease; the same may be said concerning the presence of blood, and as to the shape assumed by its corpuscles, the figure already given in connection with "chylous urine" will show that there is nothing peculiar about it, nor yet about the amœba-like movements of the blood-corpuscles, as the following easily repeated little experiment will show.

Changes occurring in freshly drawn healthy blood when placed in fluid.

A small portion of a slightly alkaline cholera evacuation was filtered off into a test tube, and having pricked my finger, a few drops of blood was allowed to fall into the fluid, with which it was immediately mixed, and a drop of the mixture transferred on a slide to the microscope; nearly all the red cells were seen to present a stellate or echinnulate appearance (Fig. lxi, 1); only a few white corpuscles were visible, and these presented a granular, more or less circular outline (2). Some, however, were spread out like an amœba (3), but no movements were seen. In the course of two hours the stellate form of the red cells had disappeared, and presented the various forms commonly seen in evacuations (4). Having been unable to see any of the white cells protrude portions of their substance, it occurred to me that, perhaps, the temperature of the fluid being only 80° was the cause; consequently another portion of the fluid was filtered and carefully warmed up to 110°, when a drop or two of freshly drawn blood was introduced. This time a very slightly granular white cell was seen to alter its form and protrude one or two vesicles from its substance (5), and draw them in again, which it continued to do for a few minutes, then ceased, becoming more granular than it was before. Others were observed to act in the same way; one pale white cell was seen to possess a very delicate filmy capsule, extending some distance beyond the contents (6); it suddenly vanished altogether, leaving a merely irregular granular heap to mark its position.

Warming the fluid favorable to the exhibition of amœboid movements in the blood-cells.

The *fourth* class of corpuscles.

The *fourth* class (it will be more convenient to consider the third afterwards), namely, the various stages of the animalculæ, was for a considerable time the subject of much curiosity, especially the kind described as presenting such activity. The fact of their being almost universally present in choleraic dejecta, and yet never, as far as I know, alluded to, except indeed that Thiersch of Erlangen could have seen one of these on the point of passing into the "still" condition, during which stage pseudopoda are incessantly projected in all directions, when he speaks of having observed actinophrys-like bodies in some choleraic dejecta which he had examined, and wondered what they were.* There was some difficulty in tracing this body to any of the described species of animalculæ. Its minute and rapid motion added to the difficulty, as well as the variableness of its shape, because although generally spindle-shaped, it may become round, triangular, or stellate in less than a second; frequently a succession of pseudopoda are seen projected in a wave-like manner, as if lashing the fluid when about to pass out of the active state. It is generally hyaline, but may be granular; sometimes a vacuole is observed, but a contractile one never. There is always a very delicate posterior filament, at first continuous with the sarcode, and a still more delicate anterior one, both retractile.

Actinophrys-like stage.

In some respects it agrees with the description of the Monad *Bodo*, but as Cienkowski, in his celebrated article in Schulze's "Archiv," distinctly states that in the amœbiform stage of all the true monads the *pseudopoda* are pointed, whilst in the amœbiform stage of this animalcule the projections are, I think, invariably rounded, so that for this and other reasons, which need not be entered into here, room may probably be found for them among the Astasiæa or Euglenæa family, so common in our tanks. The association of a cholera entozoon with the euglena, one species of which, when in its mature condition, causes the red colour observed in so many pools, and which Ehrenberg thought was the means by which the miracle was brought about of turning the waters of Egypt into blood,—the finding of precisely similar animalculæ in drains, gave rise, as may be supposed, to not a few very pretty theories, which, I regret to say, like many others, had to be abandoned altogether.

Probable family to which the animalcule belongs, and its connection with water.

A gentleman, with whose personal habits I am well acquainted, suddenly felt some griping pains with inclination to go to stool, but was otherwise perfectly healthy. The

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* The animalculæ alluded to in this Report do not in any way resemble the figures of the actinophrys-like protozoa accompanying Dr. Sanderson's account of his celebrated experiments published in Mr. Simon's Ninth Report.

Found in other than cholera stools.

motion was very scanty and very diluted, but was followed by immediate relief. It occurred to me to subject the stool to a microscopic examination, and, to my surprise, these animalculæ, both in the active and "still" stages, were present in the most perfect condition, together with numerous globules of a fatty nature, exactly similar to those already alluded to. A comparison of the figures here given (Figs. lxii—iv) with ones previously described will, I think, be sufficient without repeating that description.

The next stool passed by this individual was also a relaxed one, and microscopically of the same character, after which the motions were perfectly natural; but, in proportion as the motions became more solid, the ease with which these animalculæ could be found diminished. Many other ordinary evacuations were examined, and in fully half, after more or less careful search, they were discovered. After a brisk purgative they are frequently seen in great perfection.

The *third* class of corpuscles.

In alluding to the nature of the *third* class of bodies, namely, those found in the meshes of the fibrillated substance composing the flakes in cholera evacuation, I wish to premise that the remarks are reservedly made, as the subject belongs more directly to the pathological anatomy of cholera, which subject forms a later part of the programme drawn out for guidance in connection with this inquiry. It will, of course, be understood that the corpuscles of the former three classes are also found with the corpuscles forming this division; indeed, it is frequently impossible to separate them, especially from those amœbiform conditions of animalculæ which are seen so frequently in evacuations. This is probably the reason why so many different descriptions exist of their appearance and of the action of re-agents.

Difficulty of distinguishing these from the amœboid stage of animalculæ, because

Now, the chief statement I have to make concerning the corpuscles of this class is, that they *exhibit movements somewhat like the movements associated with the amœba.* This fact may, by very careful examination with a good $\frac{1}{8}$ of an inch object-glass, be verified by any

these corpuscles exhibit somewhat similar movements.

one accustomed to the use of the microscope in most cholera stools when perfectly fresh. A portion of the substance of the corpuscle is seen to creep out insensibly from the mass, and as insensibly return: unless the eye is carefully fixed on the body, and is already a more or less educated eye, the phenomenon is not detected, and the observer enters it as "disintegrated epithelium" in his note-book. It may perhaps be remarked that no drawing of columnar epithelium, said to be so universal in cholera dejecta, appears in this report. The reason is that its presence, to an appreciable extent, has not been observed in the contents of the intestines discharged during life; indeed, the only occasions on which I have been able to observe it quite distinctly were in discharges voided a few minutes before death, a long interval having elapsed since the occurrence of a previous stool. It was Boehm, I think, who first laid great stress on the fact of the shedding of the epithelium in cholera about 1832, since which period it has been the general opinion in Germany, with the exception of Virchow and a few others. In the well known Bavarian report of 1857 I find great prominence given to this view, modified, however, by the remark that, as a rule, only the broken down epithelium, or rather freed nuclei of such, are seen. Dr. Beale also lays great stress on the diseased condition of the epithelium, and the latest authority on the subject, Dr. Macnamara, follows Dr. Beale; indeed, it is evident that Dr. Macnamara's explanation of many of the phenomena observed in this disease is based upon a conviction of the correctness of the views advanced by these writers. It is of the utmost importance in matters of this kind, as was pointed out by Professor Parkes in 1848, not to confound the microscopical appearance of the rice-water stools passed during life with that of the contents of the intestine obtained after death. In a *brochure* which was published by him on this subject at the time I find stated:—"With regard even to the separation of the epithelium, although from the facility with which this structure is shed, even during ordinary healthy processes, it does appear probable, *à priori*,

Absence of epithelium in cholera stools.

Shedding of columnar epithelium during life advanced by Boehm.

Supported by many German writers

and by Beale and Macnamara,

but contradicted by Parkes, who maintains that it is only present after death.

that it would be largely thrown off in cholera, *there is absolutely no proof that it is so thrown off until after the death of the patient. The stools contain none, or a quantity not more considerable than is present in common diarrhœa.*"* Judging from the cholera stools which have come under my observation in Calcutta,—several hundred specimens,—I believe that not more than two out of twenty slides will contain distinct traces of columnar epithelium.

Reasons for believing that these corpuscles are not broken down epithelium nor their freed nuclei.

That these corpuscles are the remains of diseased epithelium may, I think, be disproved without any reference to *post-mortem* appearances, which I wish at present to avoid; *first*, by the fact that, under favourable circumstances, they *exhibit movements exactly analogous to those seen in the blood, pus, lymph, chyle, and the so-called "mucus" corpuscles. Secondly*, cell formations and minute flocculi, microscopically identical with these, may frequently be observed under other conditions, and from sources where it would be difficult to account for their presence were they epithelium fragments, such as in the fluid obtained by pricking a blistered surface. *Thirdly*, that even where portions of columnar epithelium are seen they will, I believe, almost invariably exhibit, no matter how much broken down the cell appears, the delicate rim or basement membrane lining the free end of the cell, believed by some to be pores communicating with the cell. The presence of epithelial fragments, when not excessive, may be readily accounted for by the process of renewal which takes place in all cells. Dr. Sharpey writes:—"The particles of columnar epithelium are undoubtedly subject to shedding and renovation. According to Donders and Kölliker, the columnar cells on the villi appear occasionally to cast off parts from their upper ends, with subsequent reparation of the loss; that is, a cell enlarging, and a second nucleus appearing; the upper and broader part with its nucleus and much of the cell contents separates, and the lower remaining portion with its nucleus grows again to the natural size." And, *fourthly*, the epithelium thus discovered in the dejecta will remain for weeks unchanged in the fluid in which it was found, showing that the action of the liquid portion of the stool is not so destructive to it as would be inferred if the numberless corpuscles seen were the result of the disintegration of epithelium which had been shed. I think there is no doubt but that these are the "peculiar corpuscles" first described by Dr. Parkes, with probably the circular, "still" condition of the animalculæ alluded to in this report, the microscopic appearance and the action of reagents coincide so entirely with the minute description given of them in the author's work. I am as yet not in a position to verify the author's belief that they are confined to any particular stage of the disease. I hope, however, to obtain more exact data on the subject in my next report.

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* The italics are mine.

Probable identity of the corpuscles with those described by Parkes as "peculiar bodies."

With respect to the nature or origin of these corpuscles and the fibrillated substance in which they are imbedded, I have not been able to disprove, nor in any way to modify, the views expressed by the writer at the time when he drew attention to them in the following extract, which may appropriately serve as the concluding sentence of this paragraph:—

Their probable nature, a modification of fibrine.

"It is in the highest degree probable that they owe their origin to effused blood-plasma, which assumes with great rapidity a low, ill-defined, and non-progressive organisation."

SECTION III.—"MICROCOCCUS."

The term "micrococcus" (*mikros* small, and *kokkos* kernel) is now pretty generally adopted on the continent by the class of writers who advocate the pre-existence of a GERM, in some shape or other, to every living thing, this germ, which may be infinitely minute, being called its "micrococcus;" whereas another class of writers, very numerous now in England as well as on the continent, maintain that the pre-existence of a germ is not necessary to the development of living objects, providing certain atmospheric, chemical, physical, and other agencies are present; the nature of the object developed depending on the relative proportion of these agencies or "forces." In short, that life is a creature of circumstances, those circumstances being of an entirely physical nature. The question of the existence or non-existence of a "germ" being of such great importance in connection with epidemics and infectious diseases generally, and its investiga-

Usual meaning attached to the term "micrococcus."

tion associated with so many difficulties, I should have preferred not alluding to the subject of this section at present, not having had time to accumulate sufficient material to enable me even to obtain a clear idea as to what changes take place, much less to attempt passing any opinion concerning those changes. As, however, it might be thought that no attention had been given to this portion of Hallier's theory,—in some respects the most important, and certainly the most difficult to disprove,—a few illustrations will be given of what has been done in the matter.

Reasons for not deferring the consideration of the subject of this section.

As already explained, the micrococcus, or germ of *cholera*, is, in the opinion of Hallier, the disintegrated spores of a special fungus, which escaping into water may be swallowed, or after being wafted by the air, adding a trifle to the "dust," according to Professor Tyndall, so prevalent therein, reach the interior of the human body, there to develop at the expense of the nitrogenous material, notably the epithelium of the intestinal canal.

Hallier maintains that the germs of cholera are the disintegrated spores of a special fungus.

It will, of course, be evident that the attempts, already described, to produce a peculiar fungus by cultivation of choleraic discharges in which bodies somewhat resembling "cysts" and "spores" existed, equally favourable conditions were at hand for the development of their ultimate elements;—seeing, however, that the fungi which then appeared possessed no peculiarity, one may conclude that either the attempts to cause the development of the particular micrococcus of cholera were failures, or that no cholera "micrococcus" existed, at least not as the germs of a fungus.

During the earlier part of the inquiry it was thought that a greater number of minute bodies of an organic nature existed in cholera stools than were found elsewhere; to this impression the mind was evidently, though unconsciously, predisposed, from the fact that the fermentation theories of cholera necessitated, to a more or less extent, the supposition that *monads, bacteria, and vibriones (Fig. lxiv) flourish to a

The statement that monads and vibriones are more plentiful in choleraic than in other discharges

---

* Irrespective of any theory as to the nature or mode of formation of these minute bodies, I have followed the example of Professor Hughes Bennett in adopting the terms "monads" when simple molecules are meant (Fig. lxiv—1); "bacteria" when the bodies are slightly elongated (2); "vibriones" when still more so; and "leptothrix" when presenting a linked appearance (4).

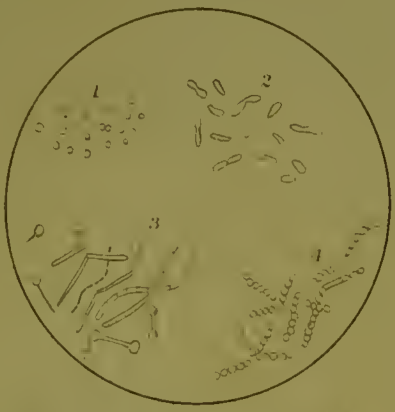


FIG. LXIV × 500

1. Micrococci 2 Bacteria
3 Vibriones 4 Leptothrix

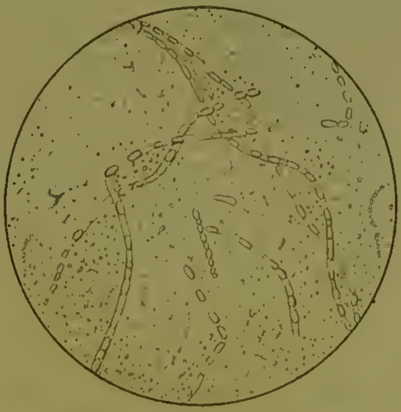


FIG. LXV [illegible]

Developed in organic solution on 2nd day [illegible]

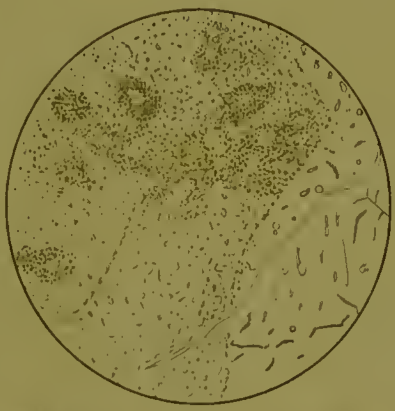


FIG. LXVI × 500

Developed in Organic Solution 3rd day
From [illegible] heap.

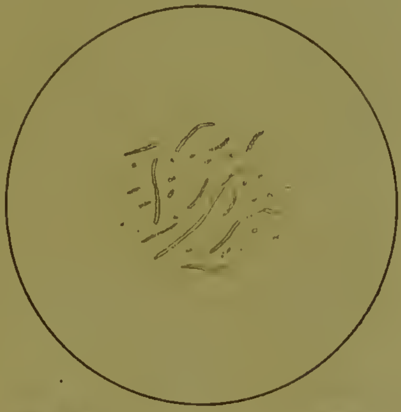


FIG. LXVII × [illegible]

Developed on [illegible] day

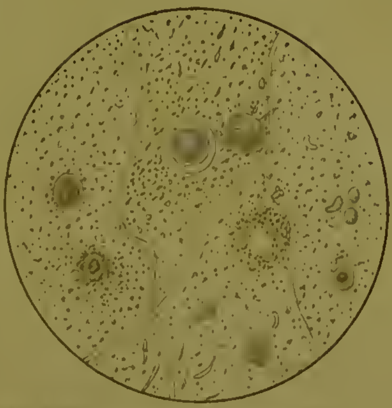


FIG. LXVIII [illegible]

Developed on [illegible] day

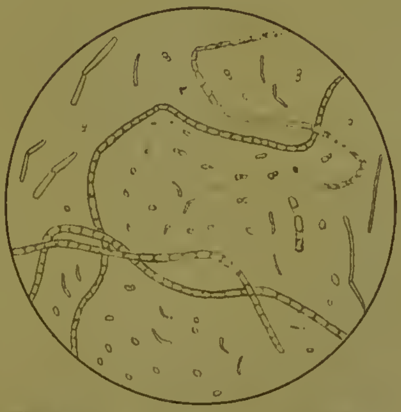


FIG. LXIX × [illegible]

Developed in Boiled Organic Solution
3rd day.

FIGS. LXIV – LXIX. DEVELOPMENTS IN ORGANIC SOLUTIONS

greater extent in this than in other diseases. Thus far I have not found this to be the case; indeed, the discharges of cholera patients, if examined immediately, do not contain such quantities of these minute bodies, especially if the stools have been voided in rapid succession, consequently have not been long detained in the intestinal canal. Neither have I been able, after repeated observations, to find that, during the decomposition of a cholera discharge, a greater number of the minute bodies associated with putrefaction were developed in it than were developed under similar circumstances, such as the amount of heat and moisture, in ordinary alvine discharges; nor have I been able to find that any peculiar growth, animal or vegetable, will proceed from the one which does not proceed from the other. On this point, however, the number of observations have been far too few—the sources of fallacy being so many—to enable one to speak with confidence, but I trust in the next report to be able to furnish more minute data concerning this matter. On an average, out of a dozen experiments undertaken, not more than one is brought to a satisfactory conclusion, which is not to be wondered at, when it is considered that the quantity of matter experimented upon does not exceed one-fourth the size of a drop of water; that this requires the free admission of atmospheric air, and that it has to be examined at least daily, for a month or more, often for hours together. Either the fluid suddenly evaporates, or the lens touches the covering glass, thus disturbing the geography of the preparation; or, which is the most frequent accident of all, and one of the most untoward, a minute spore of some fungus falls from the air upon the moist slide—germinates; the filament insinuates itself through the little air-orifice which had been made in the walls of the growing cells, and reaches the preparation, where it not only obscures the field, but alters the chemical and other forces taking place in that *droplet*, and the forms of life which had developed therein—I do *not* say spontaneously—become altered also. I have frequently observed that a slight disturbance affects the development of these minute

has not been supported by these observations.

The minute organisms associated with decomposition of cholera stools not excessive;

nor microscopically peculiar. This statement is made reservedly.

Difficulties in ascertaining the nature of minute bodies are not only manipulative, organisms; either the forms of life previously present cease to grow, being replaced by others, or the vital process becomes changed, and the manifestations of life take another direction.

but dependent to a great extent on their susceptibility to trifling disturbances.

These points will become more or less evident from a perusal of the following examples, which are intended to serve as illustrations of the method adopted in carrying out these investigations, rather than as evidence for or against any particular hypothesis.

In order to be the better able to judge of the significance of the development which might take place in substances obtained from diseased conditions, it was considered of the utmost importance that definite knowledge should be acquired of what developed in nitrogenous material, when it was known that no disease existed. With this object in view, solutions were made of various substances, to which choleraic and other discharges were added, careful notes and drawings being made of the changes that occurred from day to day.

Illustration I:—

Developments in simple solutions of organic matter.

As examples of the changes which occur in solutions of ordinary organic matter, the following experiments on watery infusions and decoctions of meat are selected on account of the simplicity of the mixture, and as illustrative of what has been stated above, namely, that slight alterations in their surroundings exert a powerful influence on the forms which living matter will assume.

Cold solution of raw meat;

(a). One ounce of carefully filtered, distilled water was placed in a test tube and a piece of raw meat (beef) about the size of a pea dropped into it, the portion being carefully removed from the centre of a fresh piece of muscle.

milkiness on the second day, due to molecules.

In the course of twenty-four hours the upper half presented a milky appearance, and there was a very thin film on the surface. It consisted of minute molecules (monads or micrococcus), together with long linked filaments of a larger size than vibriones, which were perfectly still (Fig. lxv). The milkiness of the fluid continued to increase, but the pellicle did not get much stronger, for on the third day, whilst attempting to remove a little, it broke altogether and sank in the fluid. On examination it was found to contain a few long filaments with monads and short vibriones, among which little oval accumulations or "heaps" were seen, as if composed of broken down molecules (Fig. lxvi). The next day the vibriones were considerably longer, but no other change was visible (Fig. lxvii). On the fifth day the "heaps" had increased in number; although they appear granular with a power of three hundred, they are distinctly seen to be molecules by a higher power, such as Ross' $\frac{1}{8}$ of an inch object-glass, except the central portion, which seems to be of an amorphous nature, and reflects the light differently. In contact with the particle of meat was a shreddy substance, also containing heaps, in the midst of which round cells of various sizes had developed, from a minute molecule, presenting a brighter appearance than the ones in the field generally, up to cells with a diameter somewhat greater than that of a red blood-corpuscle (Fig. lxviii). The larger ones were seen to be surrounded by a distinct capsule, between which and the contained protoplasm (which had a bluish tint) a little fluid seemed to intervene; this in certain lights presented a red tint. The hyaline substance altered its shape occasionally within its capsule, but the movement was an exceedingly slow one. In the cells a few grades smaller than the ones described, the protoplasm presented a somewhat bluish tint, but of a yellowish-blue when exactly in focus; they also seemed to possess the power of altering their form, or rather to dilate and contract very slowly.

Little "heaps" observed on the third day.

Each "heap" composed of molecules, with a central portion structureless and still.

A few molecules had increased in size on the fifth day.

Contents of the larger molecules.

These molecules kept increasing in number and size. As a rule, the larger they are, the more amorphous matter surrounds them; that is, the larger the heap. After this putrefaction set in very rapidly; no further progress occurred, but the whole became disintegrated.

(*b*). A small portion of meat was placed in distilled water as above, and thoroughly boiled for some time in a Florence flask; filtered whilst hot into a clean test

A solution like the foregoing, but thoroughly boiled.

tube (which had been previously subjected to the flame of a spirit-lamp); it was then covered, but not so tightly as to prevent the entrance of air, and placed in the same compartment as the foregoing.

Progress during the first week.

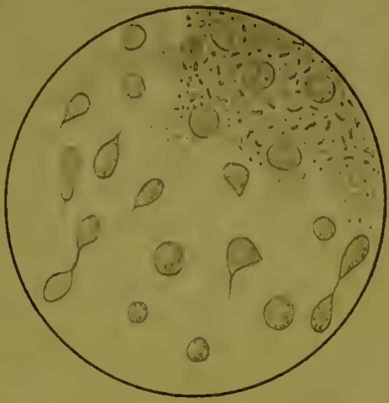
On the second day it had become milky, and presented a slight film of a somewhat similar nature to the previous one; little chains (leptothrix) interspersed throughout the field, with a few monads and short vibriones. This appearance was still more marked on the third day (Fig. lxix). On the fourth day the linked filaments were present, but the molecules (or micrococci) had increased in size, which, with the short vibriones, presented great activity. On the fifth day the milky appearance had diminished, but no change could be observed under the microscope.

Towards the end of the third week animalculæ had developed, very like the ones found in choleraic and other discharges.

The resemblance becoming still more striking by the fifth week.

Paramecium-like bodies appeared towards end of seventh week without cilia;

Little "heaps" now formed amongst the molecules, the fluid at the same time clearing up, and towards the end of the third week the slight precipitate which it contained not only presented monads, bacteria, and vibriones, but animalculæ in great numbers, which were seen at one moment elongated and very active, the next circular and still (Fig. lxx), very like the ones above described as occurring in choleraic and other discharges. In some of these, however, one, two, or three contractile vacuolæ were observed lasting about three seconds, and about three seconds absent. In addition to these, a few amœbæ were present, with no contractile vesicle; probably an earlier stage. It was then set aside for a fortnight; the animalculæ were, if anything, more plentiful than before, and when in the active or "still" condition were not distinguishable from the ones described as being found in the stools, as may be seen by reference to Figure lxxi, where, in addition, some green-coloured cells are seen. The latter were not observed to develop into anything higher, although watched carefully on a slide for two months; they simply increased in size and in number. The test tube was set aside for another fortnight, and was found to contain larger animalculæ than before, belonging to the Kolpoda family; no cilia could be made out, but a contractile vacuole was very evident. The various stages in the life history of



[illegible] ×

[illegible] solution

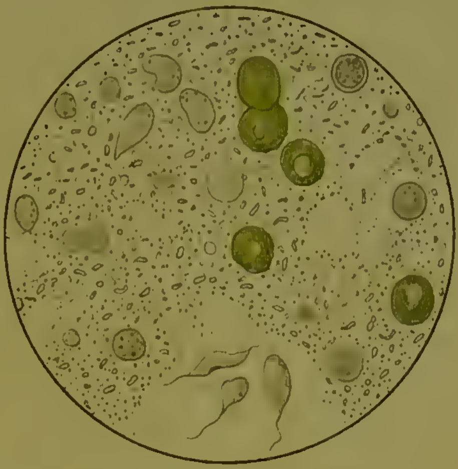
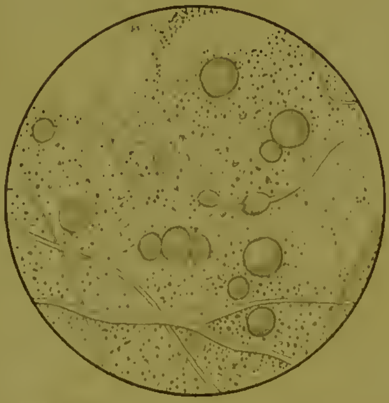


FIG LXXI. × [illegible]

As LXX [illegible] week



[illegible] ×

[illegible] solution w[illegible]

[illegible] th.

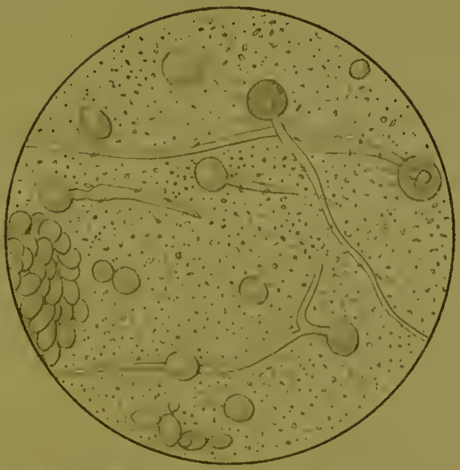


FIG LXXIII [illegible]

As [illegible] Germinating

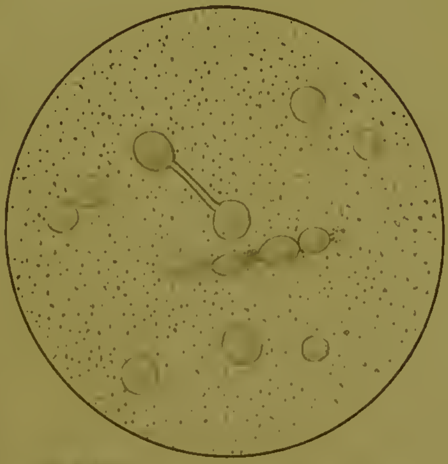


FIG LXXIV. ×

[illegible]

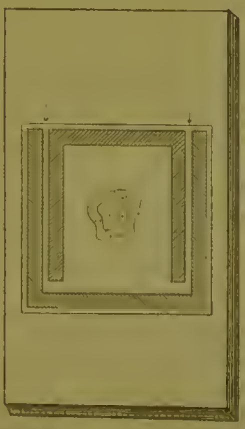


FIG LXXV. Natural Size

Dr MADDOX'S CULTIVATING CELL.

FIGS LXX LXXV DEVELOPMENTS IN ORGANIC SOLUTIONS

these animalculæ will be minutely described further on, in connection with other observations.

At the end of the third month cilia were easily demonstrable. The animalcule very closely corresponds to the *Paramecium Kolpoda* of authors.

but ciliated at the end of three months.

(*c*). A portion of the boiled solution of meat used at (*b*) was placed in another test tube, filtered with a perforated cork, in which was introduced a piece of glass-tubing bent a little more than at right angles; one end was dipped into the fluid in the tube, and the other was drawn out to a very fine point, but not perfectly closed up. This was devised with the intention of ascertaining whether expired air would produce any alteration in the forms of life which might subsequently become manifest in the decoction, as a preliminary to future experiments on organisms developed in crowded and empty rooms. With this view, the test tube was breathed into once or twice daily for a fortnight, then set aside in order that a film might have an opportunity of forming.

Third experiment with same solution.

The test tube adapted for admitting expired air.

At the end of three months the cork and glass-tubing were removed. A delicate film had formed, which on being touched sank to the bottom of the tube. The fluid was clear, free from smell, and presented no organisms when examined microscopically. A portion of the subsided film was removed by means of a pipette. It consisted of minute molecules and filaments held together by a slimy substance. Imbedded in the midst of these were a great number of yellow globules, microscopically not distinguishable from globules of oil. The appearance presented by the field is carefully delineated at Figure lxxii. They were unaffected by liquor potassæ, iodine, and dilute acids for some time; eventually, however, more or less granular contents became evident; no organic connection could be seen to exist between the globules and the filaments, and no animalcule of any family was present.

When opened at the end of three months

oil-like globules were seen in the film,

A small portion of the film was placed upon a growing slide, and a drop of the solution of grape-sugar and phosphate of ammonia added to it, so as to ascertain whether they were spores of a fungus or the "still" condition of one of the infusoria.

a portion of which was placed in a growing slide with grape-sugar.

Description of the growing slide devised by Dr. Maddox.

The particular growing slide used was the one devised by Dr. Maddox,—by far the best cell with which I am acquainted for purposes of this kind. A strip of tinfoil is cut into two U shaped pieces, one being larger than the other, so that when the smaller is placed upside down ∩, it will fit loosely inside the upright portion of the other. These are stuck in this position on a glass slide with a little varnish, over which a thin covering glass is so fixed that the only air or foreign matter which can reach the preparation must pass up the "chimney" thus formed between the inner margin of the larger strip of the tinfoil and the outer one of the smaller, as will be readily seen by referring to Figure lxxv.

Result of cultivation.

On the third day the globules were seen to have increased considerably in number, and on the eighth day germination was rapidly taking place (Fig. lxxiii).

A little gum water being added caused the central part to become clear and watery, and the protoplasm to shrink in the mycelium (Fig. lxxiv). Germination continued for a few days longer, but no more advanced stage could be attained.

Nothing developed in the water used.

(*d*). A test tube containing the water used in these observations was also set aside, but nothing developed in it.

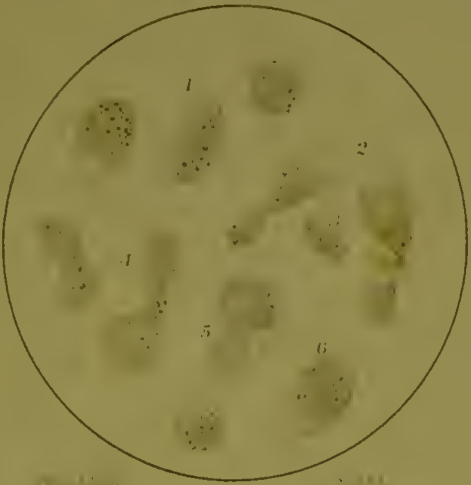
Illustration II:—

Developments in more complex solutions of organic matter, viz., normal alvine discharges.

About a drachm of ordinary fæces was dissolved in an ounce of distilled water and filtered, a portion of which was placed in a watch-glass and boiled thoroughly; a drop of this was afterwards placed in an ordinary animalculæ growing slide, both being set aside under a bell-glass.

Young paramecia developed on the third day in watch-glass, which had been previously boiled;

On the second day monads and vibriones were present in great numbers in both preparations, but on the third day they had greatly diminished in number in the watch-glass, in which, however, during the night several young animalculæ belonging to the Kolpoda family (as figured at lxxx) had made their appearance.



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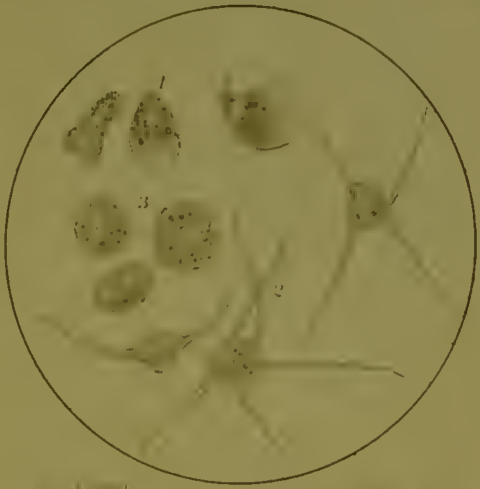

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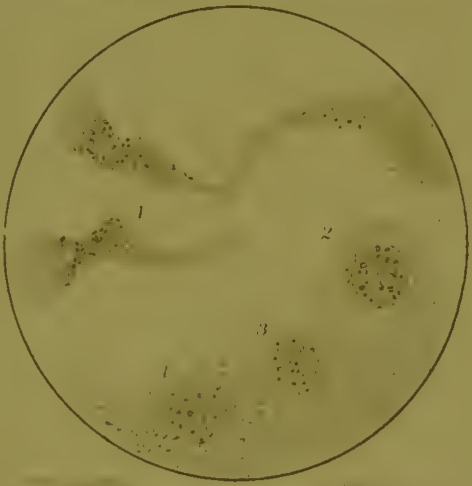

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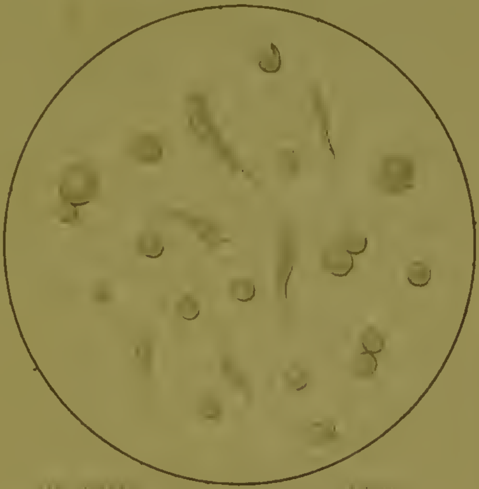

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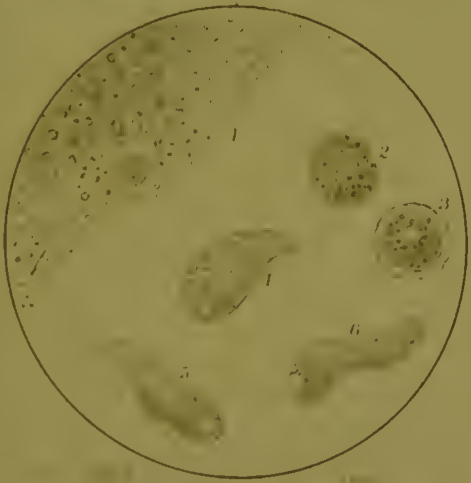

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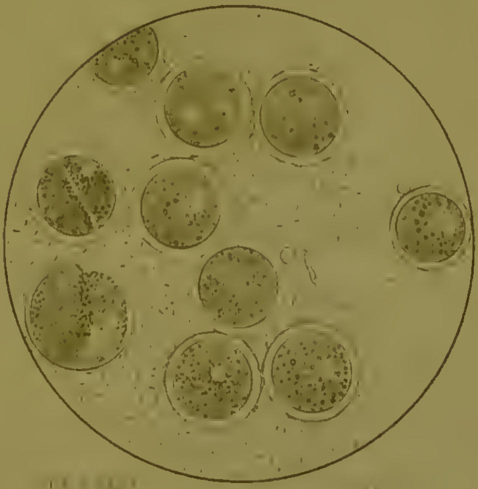

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FIGS LXXVI LXXXI DEVELOPED IN SOLUTIONS OF ORGANIC MATTER

whilst amœboid bodies appeared on the fourth day in the same solution placed in a clean animalculæ cage.

In the live-box, however, vibriones only were present as before, some of them being very long, but no Kolpodæ. On the fourth day great numbers of amœboid bodies, varying considerably in size (Fig. lxxvi, 1), multiplying very rapidly, sometimes by leaving small fragments of their substance behind (2); the portion escaping invariably from a part near the contractile vesicle, which vesicle remained bright for fifteen seconds, became puffed out suddenly, as though it had been a taper, and remained extinguished for the same period, then gradually shone again. The detached portion (3) seemed not to be merely disgorged food, for it crept about the field like its parent; it also divided into two, pretty symmetrical, halves.

Structure of the amœbæ, and their mode of multiplying.

For some time after the commencement of division, the "nucleus" is only seen in one half (4) after considerable tugging, then coming together, then separating again, each time getting a little more detached, until in the course of about two minutes the separation is complete. Frequently a mass of granules is seen to intervene, probably indigestible particles, which may adhere to either half (5), but is soon cast off, and gradually a contractile vacuole is seen to appear in the second half, which creeping along the field draws particles into its substance, and acts in every way like its parent (6).

Curious effect of adding water.

On the fifth day the fluid in the slide having somewhat evaporated, a little distilled water was added, when suddenly the hitherto more or less oval amœbæ (Fig. lxxvii, 1) commenced protruding and retracting exceedingly long processes (2), which action lasted three quarters of an hour. They then became circular and still, except that the vacuole contracted (3). In another half-hour some of them commenced to creep along the field, disgorge themselves, leaving a string of granules to mark their path (Fig. lxxviii, 1); others were observed in the course of another half-hour to become circular, with a clear halo-like ring surrounding them (2), their contents being in very active motion, reminding one exceedingly of corn in a miller's hopper. This lasted for twenty minutes, when suddenly all movements ceased; the halo and

How the granular filaments in the field are produced.

Method of disappearance of the amœbæ.

vacuole disappeared, its outline became irregular and undefined (2, 3); finally, although the eye was constantly observing it, all trace disappeared, and no distinction could be observed between other molecules in the field. The remaining amœbæ seem to have undergone the same change, for when the eye was removed from the particular one described, none could be found, except a few empty-looking ones. I have frequently observed exactly similar phenomena occur in the so-called salivary corpuscles. No further change occurred in the slide, nor was there a return to the former condition during the succeeding week.

Similar phenomena observed in salivary corpuscles.

In the watch-glass the animalculæ continued to increase and multiply, but other kinds did not appear. The glass was held over a spirit-lamp and the liquid boiled, in order to see if out of their dead bodies others of the same or of another kind would appear; but none did, and at the end of a fortnight the experiment was brought to a close.

State of the solution in the watch-glass.

Illustration III:—

The ordinary stool, to which allusion was made at page 33 as containing such quantities of the animalculæ in the "still" and active condition, was kept under observation for six weeks.

Cultivation of the ordinary stool which contained the animalculæ.

A slide was taken and two minute portions were placed side by side, a distance of about half an inch intervening, and circular covering glasses applied of the same diameter.

Two preparations on one slide

During the first, second, and third days the changes which occurred were alike in the two preparations. The oil globules gradually disappeared, the circular, "still" condition of the animalculæ became at first granular, ceased presenting the amœboid projections, the latter being frequently not retracted, but trailed along as they rolled under the glass; the general appearance of the altered slide being represented at Figure lxiii, the earlier condition having already been described, and is figured at lxii.

underwent similar changes until

The movements of the active little entozoon became more and more sluggish; at the same time it became granular and circular, and finally disappeared altogether, probably passing into the "still" condition, which also gradually disappeared. The two preparations now assumed different appearances.

the fifth day.

Filaments of oidium lactis appeared in one,

together with the little "heaps," in the midst of which yeast cells appeared.

(a). On the fifth day some fungi were seen to develop in one of the preparations, which may be designated—*a;* long filaments of oidium lactis, as figured at xxiii, commenced spreading over the entire preparation, and in the midst of the molecules (which had also undergone various stages, as already described in the first illustration) little "heaps" were forming of precisely the same microscopical characters as are given at page 40 and other places. On the sixth day a few molecules in the midst of the heap had increased in size, and on the eighth day nearly every heap was covered with yeast cells, in conjunction with very minute anguillulæ (?) (Fig. lxxix).

No further changes.

The oidium lactis disappeared entirely in the course of a few days, but no other changes took place for a month, except that the yeast cells degenerated also.

The other preparation contained no trace of fungi.

Molecular aggregation occurred on sixth day,

on which corpuscular bodies appeared next day.

(b). The portion under the other covering glass showed no evidence of fungal development, nor yet yeast cells or anguillulæ. On the sixth day accumulations of perfectly motionless molecules had formed, especially near the edge of the glass, each heap possessing, as usual, a kind of central kernel with a more or less protoplasmic appearance; the molecules forming the peripheral part of the heap being quite as active as the molecules elsewhere. On the seventh day these heaps were crowded with cells of all sizes. Some of the molecules were larger than formerly; the greater number of the cells, however, were from about the size of a red blood-corpuscle to four times that size; the contents of the larger ones being more distinctly molecular than that of the smaller, otherwise no difference could be established between them. It is, however, particularly to be noted that the steps from

the minute molecules to the smaller sized corpuscles were by no means so gradual; it did not appear as if a sufficient number of molecules of the intervening grades existed to enable one to say that the large corpuscle was simply a developed molecule. On many occasions great pains were taken in order to try and settle this question, but each time, although after the formation of heaps molecules have been seen to become, so to speak, swollen, suddenly little corpuscles appeared with undefined outline twice or three times the size of the molecules, and in a few hours the field is crowded with animalculæ. The difficulty of ascertaining this point is due to the *suddenness* with which these changes take place. After watching a certain little heap for several hours without any appreciable alteration having occurred, the eye becoming tired, it is allowed to stand unobserved for an hour or two. On returning, probably everything is changed; either the particular heap watched has become altered, or some other heap in the preparation has been more advanced and discharged the elements of life which it contained, and these animalculæ rushing about the field knock the watched little heap over, disturbing its entire geography. This is precisely what occurred in the preparation now under notice. It had been watched all day in order to ascertain whether the swollen molecules would swell still more in the course of the day, but they did not, or (2) whether some of them would coalesce and form one ovum, as believed by Dr. Bennett; neither did I see this, nor could I learn that the half slimy-looking kernel surrounded by molecules had acquired a clear "nucleus" and formed *one* body, as advocated by Pouchet, for no appreciable change occurred during those twelve hours. But when examined on the next morning, twelve hours after, a great number of corpuscles or cysts were present in the midst of these "heaps," and several, what seemed to be young *paramecia*, rushed about in all directions (Fig. lxxx). Whatever it was that had taken place, it did not seem to me that one heap had given rise to only one cyst, because three, four, or more of various sizes would be seen on the surface, or what seemed to be the surface of a heap (1).

A gap seems to exist between the diamater of molecules and the smaller corpuscles.

Almost simultaneous appearance of the corpuscles and the animalculæ.

The great difficulty of following out these changes.

One "heap" appears to give rise to more than one cell.

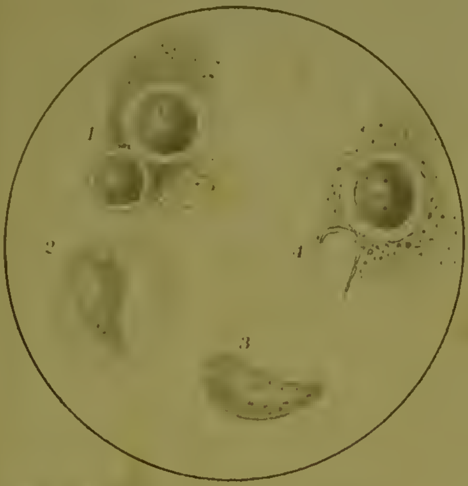

FIG. LXXXII. [illegible]
[illegible] Larvæ [illegible] out of
[illegible] encysted condition

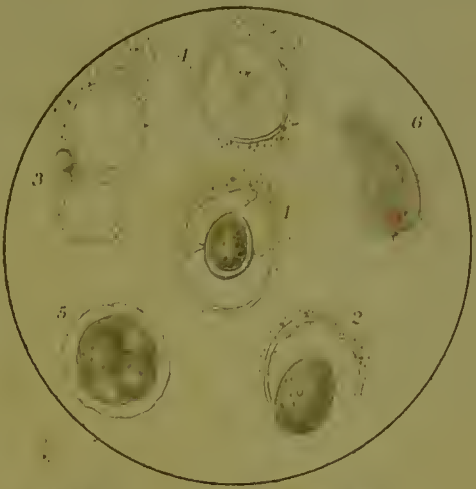

FIG. LXXXIII [illegible]
1 [illegible] 2 [illegible] Animalcule
3.4. Empty [illegible] 6. More highly developed
Larvæ [illegible] 5 Cyst containing [illegible]

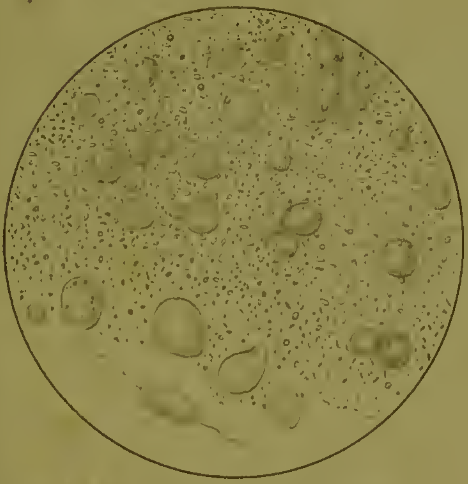

FIG. LXXXIV [illegible]
[illegible] active [illegible] of the Animalculæ
[illegible]

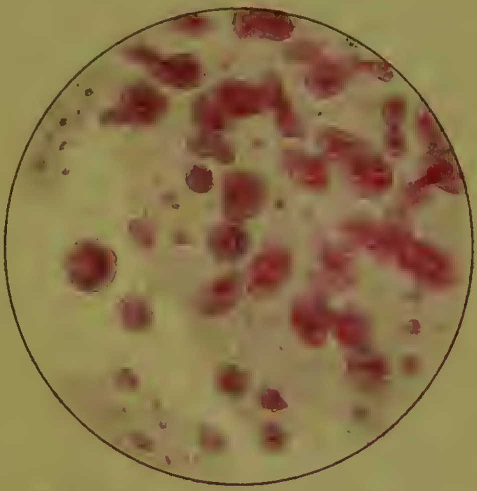

FIG. LXXXV. [illegible]
[illegible]

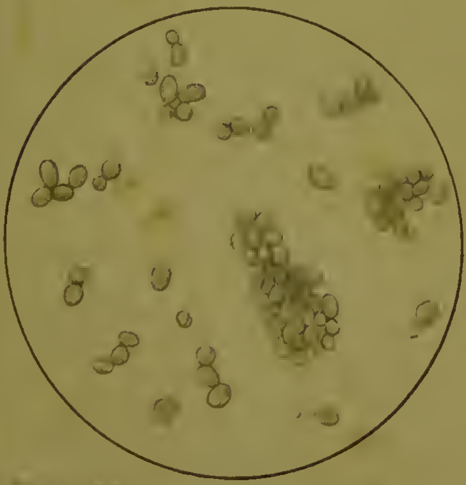

FIG. LXXXVI [illegible]
[illegible]

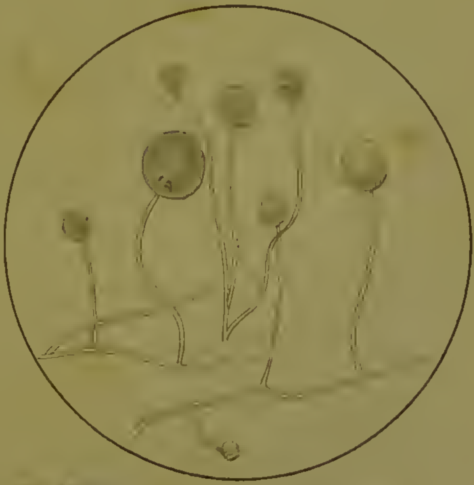

FIG. LXXXVII [illegible]
Develop[illegible]

FIGS LXXXII LXXXVII DEVELOPMENTS IN ORGANIC SOLUTIONS

Stages in the development of this animalcule.

Movements of molecules in the cell, and gradual appearance of vacuole.

In a few hours the mass spins off-and-on, like a top.

Contents escape as a non-ciliated infusorium;

after a time it becomes encysted.

Movements of molecules recommenced.

I am ignorant as to what occurred between the stage of molecular aggregation and the development of the smaller sized cysts. A few of the after changes, however, were more easily followed. A slow rolling kind of motion commences in the mass of granules, in the midst of which a clear space or vacuole becomes more and more distinct (2), at first non-contractile, then it suddenly goes out and does not return for two or three minutes; gradually these intervals become shortened: contraction and dilatation occurred pretty regularly at intervals of 15 seconds. In a few hours it commences to spin like a top without in the least altering its position. Then it stops, its nucleus becomes extinguished, and the body appears pretty much as it did at first. After a shorter or longer period the action recommences, and eventually it becomes elongated, gets out of the heap into the fluid, and rushes about as if locomotion were nothing new to it. No cilia can be seen, nor any trace of nucleus, merely a contractile vesicle at the broader posterior end, with granules and molecules universally distributed (3). In those parts of the field where the fluid is rather thick, it creeps along something after the manner of an amœba (4). On the next day, the eighth, several were seen to move very slowly and to become circular and still. They became surrounded by a clear hyaline capsule, and the vacuole again disappeared. A few hours after this the field presented the appearance shown at Figure lxxxi. Some were perfectly still, and had no contractile vacuole; in others the molecular contents showed active movements, with or without a vacuole; in some two vacuoles were visible, not contracting simultaneously, and in such cases there seemed to be two centres of movement—two irregular masses seemed to move within the cyst. The evidence of division was frequently more marked, a regular line of separation existing, and in others two oval bodies are seen to revolve within the capsule. In the drawing the remains of two cysts are also evident. In order to know exactly the phenomena associated with the escape of the animalculæ, the pair delineated at Figure lxxxii, 1 were selected and continuously watched.

The escape out of the cyst.

A rolling movement commenced among the molecules of the smaller one, which increased, until at last each molecule seemed to dance past the other. The vacuole went and came rapidly, lasting about six seconds, and in the course of another six seconds returned, then became perfectly still; movement recommenced and stopped in the same way. Thus it acted for some time; each time, however, the outline of the contents became more evident, and the cyst became more and more distended, finally ruptured (2), and the body rolled out. It was evidently not yet free, and its outline was indistinct. Very active movements were now set up, pseudopoda pushed out in every direction, and it was seen to be still surrounded by a very delicate sac. By continually turning itself about, this film became much distended, and so transparent that its form was distinctly visible (3). At last the pellicle became so attenuated that it escaped without trouble (4). The same process takes place when the animalcule has divided into two or four.

Movement of molecules followed by rupture of cyst.

Escape of infusorium enclosed in a sac,

which after a time gives way, and the animalcule is free.

In the encysting process which follows, the cysts seem to become thicker, and a little fluid is frequently seen between the inner lining of the cyst and the delicate sac* which surrounds the animalcule. Frequently such cysts are seen to have become ruptured some time before the escape of the contents (Fig. lxxxiii, 1), and it not seldom happens that the latter after its escape does not rupture the thin inner capsule (2), but remains perfectly quiet for two or three hours. Old cysts persist for some days after being forsaken by the infusoria (3), and not infrequently the latter has left a few granules to mark its former abode (4); two or three may also develop in these thicker kinds of cysts. The size of the cyst bears no positive relation to the number of bodies it may contain; a comparatively small cyst may contain four embryos, allowing of active movements, as existed in the one delineated at 5.

The encysting process repeated many times; segmentation into two or four may or may not take place during each encysting process.

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* This, according to some writers, is the "cyst," outside which is the "cell," surrounded by the hyaline gelatinous "veil."—(*schleier.*)

In the course of a month cilia and a nucleus evident.

At the end of a month numbers were seen distinctly ciliated; a nucleus became developed, as well as a contractile vacuole, and a current was established at the anterior portion of its body, so that particles were drawn towards it (6).

Illustration IV:—

Developments in cholera stool.

The fresh dejection of a cholera patient was examined almost immediately. The sediment was found to be composed of a slimy substance dotted with granules and molecules, intermixed with a great number of more or less circular bodies, some hyaline, some granular, many of which appeared to me to be the still condition of animalculæ, as already alluded to, together with several euglena-like bodies, disporting themselves in the more fluid part of the field. A careful sketch of these objects is given at Figure lxxxiv. There were plenty of monads and bacteria in the field, but the vibriones were exceedingly small and short.

Its early condition.

Effect of carmine solution.

A solution of carmine in glycerine after prolonged action seemed to stain everything in the field to the same extent; the varying density of the colour seemed to depend entirely on the thickness of the layer; that is, a larger amount of colouring matter was present when the layer was thick (Fig. lxxxv).

Evacuation placed in growing slide.

(a.) A minute quantity of this stool was placed in the Maddox growing slide already described. During the first two days the objects became more and more disintegrated, until on the third day not a trace existed of the circular bodies and animalculæ previously existing.

On the fourth day a few creamy-looking spots were seen at the edge of the preparation, consisting of innumerable molecules (monads) manifesting very *great activity,* together with some short vibriones.

Disappearance of the corpuscles, &c., and formation of a white margin by the fifth day.

This condition had increased greatly by the next day, the creamy appearance having extended to the entire margin of the fluid, to the extent indicated by the dark outline of the preparation in the figure of the Maddox slide at lxxv.

Nature of this white margin.

In the midst of these molecules little *heaps* were seen to form, in which no motion was evident, nor yet any definite structure, but amorphous granules, around which, and above and below, myriads of monads and short vibriones played. Some slight distance from the margin an opaque line, consisting of unusually active monads, was seen separating the creamy ring into an inner and outer portion, but no distinction could be observed between the appearance of the molecules of one side from that of the other, except that in the outer the *heaps* were more plentiful.

The monads, &c., in the central clear space had become perfectly still, and no *heaps* had formed amongst them; towards evening the line seemed to have spread on either side, as the whole creamy ring became as thickly studded with molecules—consequently opaque—as the narrow line was in the morning. The circular cells seem to have disappeared altogether.

Some molecules near the "heaps" enlarged,

On the sixth day not a single molecule quivered. The creaminess of the margin had slightly diminished, but the "heaps" were still present, rather more slimy-looking, not so regularly circular, but frequently elongated and straggling. The diameter of some of the molecules in contact with the mass had increased.

but a fungus crept in and spoilt the preparation.

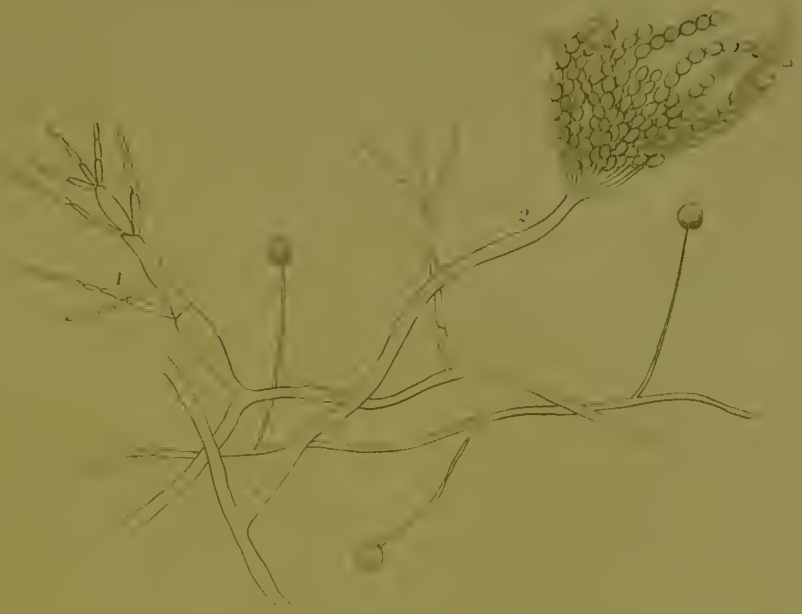
A mycelial filament was now seen to insinuate itself from without into the preparation, having crept up the "chimney" of the growing slide, and the further development in or about the heaps came to a stand-still, although watched for a month. The mycelium spread in every direction, and gave rise to yeast cells.

Slide prepared as last with addition of cultivating fluid developed penicillium.

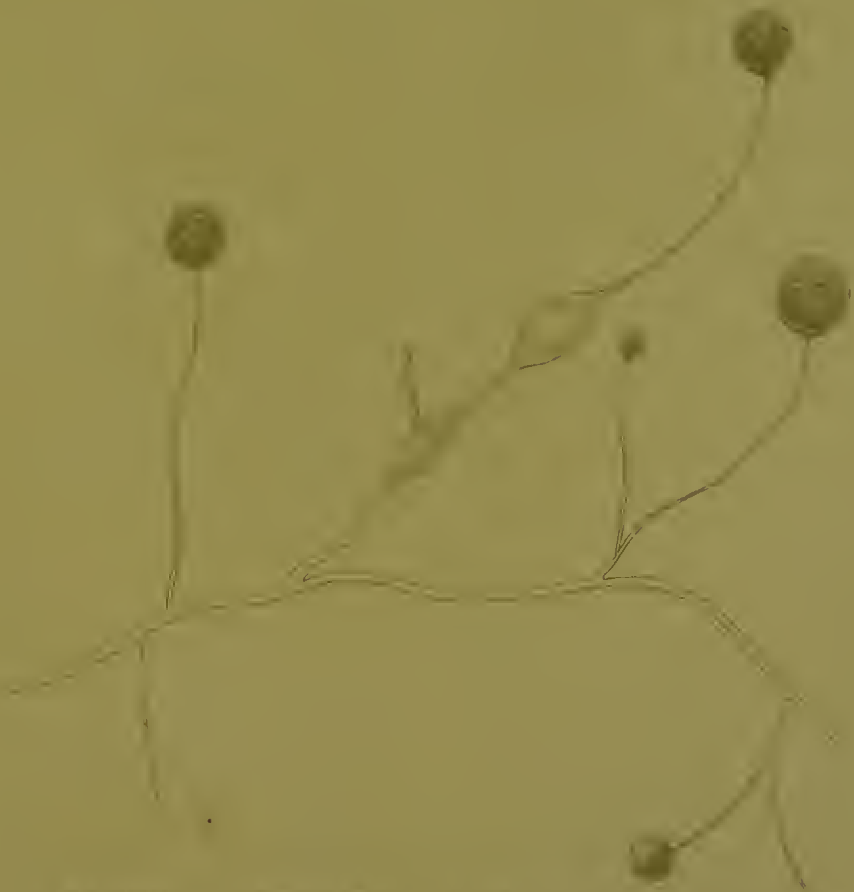
(b). A similar slide containing a drop of this stool, to which a little of the growing solution of grape-sugar and phosphates was added, went through the same stages as the foregoing, and developed into *penicillium*, as in Figure xix, 2.

A similar preparation without a covering glass

(c). A portion of the same preparation without a covering glass was preserved in a moist chamber. On the third day a white speck was seen in the surface consisting of innumerable "yeast" cells (Fig. lxxxvi), with some filaments branching in all directions. On the fourth day



1 [illegible] 2 [illegible]



[illegible]

FIGS. LXXXVIII IX DEVELOPED IN CHOLERA STOOL

Produced penicillium associated with a mucor-like form of fructification.

Acid reaction of preparation.

tufts of *penicillium* had developed—two varieties (Fig. lxxxviii)—*P. Glaucum* (1), and *P. Viride* (2). This continued until the ninth day, when a few of the filaments springing up in the midst of the *penicillium* were tipped with a dewdrop-like dilatation excessively delicate—a mere distended pellicle. In some cases they seemed to be derived from the same filament as others bearing the ordinary branching spores of *penicillium*, but of this I could not be positive. This kind of fructification increased rapidly, and on the fourteenth day *spores* had undoubtedly developed within the pellicle (Fig. lxxxvii), just as had been observed in a previous cultivation (page 13), precisely similar revolving movements being also manifested. The reaction of the liquid portion in the cell was slightly acid, and became very much more so in the course of a month. No further change took place, except that the capsules became rather thicker, but never so resistent as to withstand the action of a drop of water, spores being instantaneously set free by it.

Seeming germination of the enclosed sporules.

In not a few cases a chain of spores, or sometimes delicate filaments, seemed to escape from these cysts, as if the spores within had germinated; which indeed must have been the case, unless they had fallen from a tuft of penicillium and adhered to the capsule. In other cases dilatations (macro-conidia) appeared in the filaments, and even from these a chain of spores was occasionally seen (Fig. lxxxix).*

(d.) A small portion of the evacuation was placed on an ordinary slide with a covering glass. It went through the same process as was described in connection with Maddox's slide (*a*), and eventually yeast cells were produced as at Figure lxxxvi, but nothing further.

A preparation like the foregoing placed apparently under the same conditions

(e.) A similar slide placed in the same moist chamber presented similar changes as the foregoing for the first four days. It was not examined on the fifth, but when placed under the miscroscope on the sixth day, representatives of the *kolpoda* family, both active

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Probable connection between *penicillium* and *mucor.*

* In connection with the appearance of this mucor-like fructification in such intimate connection with penicillium on this and on other occasions, although merely an approach to the "cholera fungus" of Hallier—a fructification resembling it much more closely, if not identical with it, having been obtained under like circumstances from ordinary excreta—it must be allowed that it speaks very strongly in favour of the view so firmly advocated by this mycologist of a generic connection between *penicillium* and *mucor.*

produced young paramocia.

and encysted, had made their appearance in great abundance; the various stages in their subsequent development corresponding precisely with what has already been described in connection with experiments on ordinary excreta.

Experiments conducted with other substances.

In some cases the admitted air had first to pass through a heated tube.

Serous fluid, blood, and urine, from persons affected with cholera, as well as from other persons, have been in like manner subjected to systematic and continuous observations, the air in some of the experiments having been made to pass through a red-hot tube before its entrance into the chamber in which substances under examination had been placed, as adopted by Professor Tyndall, in order to destroy the minute *atomes* of organic matter which, according to this gentleman's researches, will pass through sulphuric acid or caustic potash undestroyed. The particulars of these observations are reserved for the present, the results being such that no benefit could be attained by giving them in detail. It is nevertheless hoped that the foregoing illustrations will sufficiently explain the methods adopted in investigating the subject of this section. The description of the changes which occurred during the cultivations has been condensed as much as possible; more so than would be allowable, were they intended to establish any particular fact.

Statement of results postponed.

Lesson to be derived from the last series of illustrations.

A not unimportant lesson is, however, conveyed by even the comparatively few experiments which have been conducted, namely, that, in spite of more than ordinary care, very different forms of life will make their appearance in substances which are derived from the same source under conditions which *seem* to be identical, and that too in very simple mixtures. Consequently, the greatest caution must be exercised in estimating the importance or otherwise of any peculiar manifestations of vitality which may be observed in substances associated with disease.

Summary of conclusions drawn from all the observations.

The results of the investigations referred to in this report may be thus summarised:—

"Cysts."

1. That no "cysts" exist in choleraic discharges which are not found under other conditions;

Sporangia.

2. That cysts or "sporangia" of fungi are but very rarely found under any circumstances in alvine discharges;

Special fungus.

3. That no special fungus has been developed in cholera stools, the fungus described by Hallier being certainly not confined to such stools;

Animalculæ.

4. That the still and active conditions of the observed animalculæ are not peculiar to this disease, but may be developed in nitrogenous material even outside the body;

The flocculent deposit, and Parkes' "peculiar corpuscles" not composed of epithelium.

5. That the flakes and corpuscles in rice-water stools do not consist of epithelium, nor of its *débris*, but that their formation appears to depend upon the effusion of blood-plasma; and that the "peculiar bodies" of Parkes found therewith correspond very closely in their microscopic and chemical characters, as well as in their manifestations of vitality, to the corpuscles which are known to form in such fluid; these are generally, to a greater or less degree, associated with blood-cells, even when the presence of such is not suspected, especially as the disease tends towards a fatal termination, when the latter have been frequently seen to replace the former altogether; and

The appearance and amount of vibriones, &c., not exceptional, but may be so in their nature.

6. That no sufficient evidence exists for considering that vibriones, and such like organisms, prevail to a greater extent in the discharges from persons affected with cholera, than in the discharges of other persons, diseased or healthy; but that the vibriones, bacteria, and monads (micrococcus) may not be *peculiar in their nature*, for these *do* vary, may not be the product of a peculiar combination of circumstances, and able to give origin to peculiar phenomena in a predisposed person, is "not proven."

Conclusion of Part I.

In bringing this part of the report to a close, I wish to express my sincere thanks to Dr. John Murray, Inspector General of Hospitals, Indian Medical Department, who has, week by week, watched the progress of these experiments, and given such practical advice and assistance as his long study of the subject peculiarly enables him to do. I also desire to tender my thanks to Dr. Brougham of the Presidency General Hospital, and to Dr. Baillie of the Chandney Hospital for the facilities which were placed at my disposal for obtaining the *matériel* requisite for these examinations; as well as to Dr. Norman Chevers, Principal of the Medical College, for permission to make use of his private library, as well as the library attached to the College.

PART II.

REMARKS REGARDING THE SOIL, &c., OF CERTAIN PLACES IN RELATION TO PETTENKOFER'S THEORY OF THE CONNECTION OF CHOLERA WITH THE VARIATION IN THE LEVEL OF THE SUBSOIL WATER.

Seeing that Professor Pettenkofer's observations extend over a period of 16 years, during which constant observations have been taken by him of the water-level in various parts of Munich and elsewhere, it will be at once evident that the short period which has elapsed since the commencement of this investigation in India cannot enable one to have formed but most indefinite conclusions on the subject. Accustomed as the Bavarian Health Officer has been for many years to much deep thinking on the subject, it is frequently difficult for less trained intellects to follow his exact meaning on all points, as the theory is by no means so simple that "he who runs may read."

During the last year a work* was issued by him embodying the result of the labours of previous years, in which the views already advanced are maintained with even a greater conviction of their truth than before.

The principal features in Pettenkofer's theory.

The main points in the theory are—(1) there exists a specific cholera poison, which (2) reaching the soil undergoes various stages of development, providing (3) that a certain amount of moisture is present; (4) should the ground not possess the requisite amount of moisture, be either too dry or too wet when the poison is placed therein, the latter will retain its vigour until the requisite conditions return; but (5) these having returned, it does not follow that an epidemic will forthwith break out, unless (6) certain meteorological conditions are present (the precise nature of which is unknown), and especially there must be (7) a predisposition to the disease in persons coming within the area in which the poison is found.

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* "Boden und Grundwasser in ihren Beziehungen zu Cholera und Typhus." Von Max v. Pettenkofer. München, 1869.

The season during which cholera is worst in Lower Bengal does not correspond with worst period in the Upper Provinces.

The reason why the period when cholera usually breaks out in the Upper Provinces does not correspond with the period in which it is at its height in Calcutta and in Lower Bengal generally, is, according to Dr. Pettenkofer, due to the fact that in the former place the ground for the greater part of the year is too dry, there being no rain, and the water being commonly many feet from the surface; whereas in Lower Bengal when the rains set in cholera ceases—the ground becomes too wet. Cholera is worst in the latter when the water-level is at its lowest, namely, about April; whereas in the former cholera is at its worst when the water-level is rising or about subsiding (August and September), so that, I presume, the Munich Professor would explain the reason why cholera is endemic in Bengal, and only epidemic in the Upper Provinces by the fact that the wet season is much shorter in its duration than the dry, consequently the conditions necessary for the development of the poison occur only during short periods, whereas the same conditions affect Lower Bengal in a different way, giving rise to short periods of exemption, instead of the short periods of attack, consequently the inhabitants of the "Ditch" are more exempt from cholera when it overflows with water.

During the rains it is too wet for the development of the poison in Bengal, and at other times it is too dry in the Upper Provinces.

It must also be borne in mind that a local fall of rain is not in all cases the only cause of variation in the height of subsoil water, for an adjoining river may rise or fall, irrespective of local conditions, and perhaps give rise to an alteration in the amount of moisture present in the soil. In a great number of instances, however, the level of the water in a well adjoining a river is considerably above that of the river, as a non-porous, clayey layer may dip towards it, thus confining the water to its bed. Again, heavy falls of rain on distant hills may affect the level of the water in the plains, should an impermeable stratum extend from the one to the other, over which water might flow. These and many other such facts connected with the geology and the topography of a place must be carefully considered before any opinion can be formed of the

Local rainfall not always a true criterion of the amount of soil moisture.

correctness of the views advanced by this distinguished Professor.

The cholera-germ does not develop in water, nor in the human body,

It will be seen from the foregoing that the poison is considered not to *develop* in water, which is contrary to the commonly received opinion, nor does it multiply to an appreciable extent in the intestinal canal, the human body being merely the *stage* upon which this actor plays its part. The poison requires a special *nidus* in which to multiply and to develop into infecting matter. This Pettenkofer traces to the soil, especially to alluvial soil, which, being so exceedingly porous, allows free interchange between the air in its interstices and the air above, as well as being subject to a great variation in the amount of water which it contains.

but in the soil.

Tendency of cholera to attack places situated along rivers.

Whilst marking out on a map the places suffering from cholera, he was particularly struck with the predisposition it seemed to manifest for following the natural water-courses of the country, rather than the usual routes of traffic. In the former, the places of attack were pretty regularly situated, whilst along the roads for intercommunication, the affected places show great irregularity, cholera spreading only in those parts in which the soil was of an alluvial nature, although quite as many opportunities existed for the dissemination of the poison by means of intercourse in the places never attacked as in the less fortunate localities.

The cholera-germ supposed to be of the nature of a ferment.

The cholera-germ, as described by Pettenkofer, may be defined as a specific *leaven*, requiring earth, consisting of organic matter and salts, with a certain amount of water for its development to infectious matter, just as other ferments require certain special substrata and moisture before it manifests its action. If ordinary leaven be added to sand no action takes place; if it be added to dry flour, it does not spread beyond the immediate vicinity in which it was placed; but if the flour be moistened "the little leaven leaveneth the whole."

The poison may be disseminated in two ways, viz.—

The question naturally occurs—By what means does it get into the human body after being thus developed in the earth? To this Pettenkofer replies: There are two ways by which substances may arise from the ground, even from a great depth :—

water and air.

By means of (1) the water, and by means of (2) the air contained in its interstices.

Possibility of the air becoming contaminated by substances rising from below.

Numerous illustrations may be produced of the possibility of substances, perceptible to the olfactory nerve, making their way upwards from considerable depths, such as when a sewer bursts, or an escape occurs in a gas-pipe. Frequently this fact is not observed where the mischief has taken place, but in a house, perhaps, some distance from it, the warmth of which, should it not stand on an impervious layer, attracts the disengaged matter like a chimney, and the house acts as an escape-pipe for a noxious gas. Were it ever conclusively shown that cholera depended upon some fermenting process taking place in the ground, which had been originated by some of the poisonous material being placed in a soil adapted for its development, it would follow that in India large substantial buildings would be safest by day, being cooler than it is without; but, unless the flooring were made impervious to air, it would be the most unfavourable at night, being warmer, consequently the native's hut approximating more closely to the temperature of the air ought to be more exempt from cholera.

Tendency of foul air to enter heated rooms with permeable floors.

Water, a vehicle for the dissemination of cholera.

Although cholera is not considered to acquire its property of infection by being developed in water, still water as well as air may act as a vehicle conveying the infectious matter from the ground, consequently this theory in no way affects the importance to be attached to the value of obtaining water from a pure source; indeed it speaks very strongly in favour of obtaining it from places as far removed as possible from human habitations.

Buchanan's explanation of Pettenkofer's theory.

Particular attention has been drawn to this subject very lately* by Dr. Buchanan, one of the several distinguished Sanitary Officers whom Mr. Simon has gathered around him at the Public Health Department of the Privy Council. While allowing that there is a connection between the disease and the level of the water in the wells,

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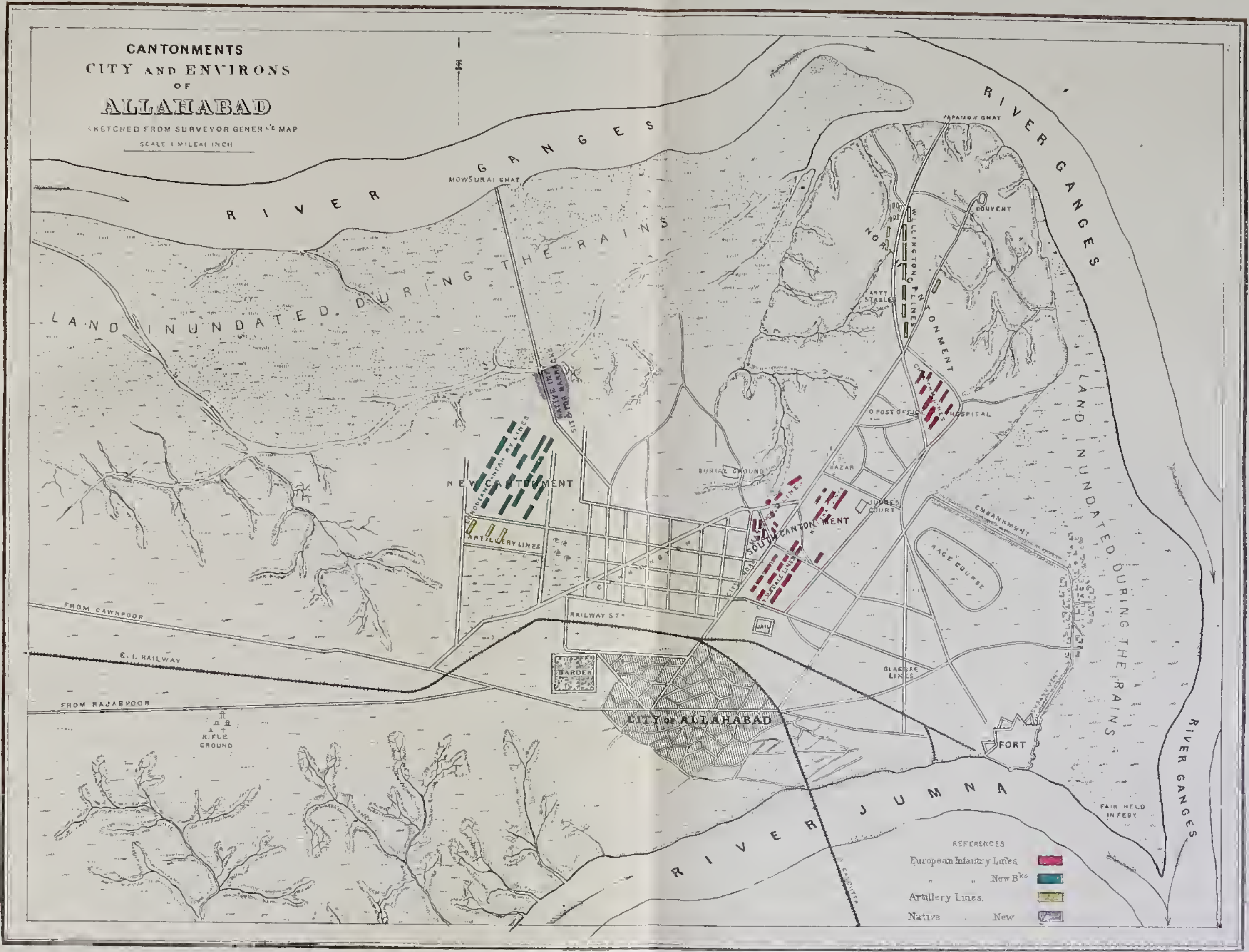
* *Medical Times and Gazette*, 1870.



Lithographed in Colors at the Surveyor General's Office, Calcutta, August 1870.

said to exist between the spread of cholera and the state of the ground water.

I now proceed, in as few words as possible, to give an account of my visit to the places affected with cholera in the North-Western Provinces during the severe epidemic of last autumn.

ALLAHABAD.

Arrival at Allahabad.

I arrived at Allahabad towards the end of August, in accordance with the instructions I had received, in order to accompany the Sanitary Commissioner with the Government of India in his tour through the cholera affected districts, and thus be able to avail myself of his advice and direction.

Seeing that our visits to the various places, to be hereafter alluded to, were necessarily of short duration, it was impossible for me to obtain more than a very superficial knowledge of the geography of a place extending over such a wide area as Allahabad does.

Relation of Allahabad to rivers.

Situated in the angle formed by the junction of two rivers, the Ganges and Jumna, it was thought not improbable that the high or low condition of these rivers might materially affect the level of water in the wells, seeing that many acres of land are swamped during the rains, the station being almost surrounded by water, as a glance at the accompanying map will show.

The level of the subsoil water nearly 30 feet below that of the Jumna.

This, however, was ascertained by Dr. Bow not to be the case, at least as far as the Jumna was concerned, the water in the wells being nearly 30 feet below the level of the surface of the Jumna.

Extent of variation of level.

The average depth of the wells from the surface, as examined by Dr. Chalmers and myself, was found to be from 50 to 65 feet. The average variation in the level of the water between the dry and the wet season is about 10 feet, whereas the Jumna varies to the extent of 30 feet or more under ordinary circumstances; nor does the alteration in the water-level of the one correspond with the variation in the other, and a consecutive fall of rain of 12 inches will not raise the level of the water to the extent of more than one or two; a great portion, doubtless, finding its way into the river before getting into wells, especially after the first

falls of rain, when the ground does not permit of such free percolation. It cannot, therefore, be said that the amount of subsoil water at Allahabad is materially affected by the rivers which bound it on either side; nor will it be safe to judge of the extent of moisture present in the subsoil by the registration of the well water alone; the *rainfall* must also be taken into account, as the latter at Allahabad appears to influence the condition of the soil more than the permanent subsoil water.

The wells not affected by the rivers.

Condition of soil chiefly affected by the rainfall.

The soil here is of a sandy, clayey nature, intermixed with layers of kunkur.

In the hot weather extensive fissures are to be observed everywhere in the ground extending to great depths, and exceedingly permeable to water. On subsequent examination, it was found, when dried in the sun, to be solid to the extent of one-half, the other half being interstices filled with air.

Porous nature of the soil.

In order to have a more precise knowledge of the extent of the porosity of the soil upon which the various barracks have been built, and which are said to vary in the degree of their liability to cholera, although in other respects apparently as like one another as it is possible for buildings to be, and the sojourners therein subject to precisely the same influences as regards food, clothing, and water, it was thought that perhaps some clue could be obtained by ascertaining the extent to which the soil beneath the buildings was permeable to the air below. General Travers, V.C., immediately permitted samples of this soil to be obtained, which on my return to Calcutta were subjected to the following treatment:—A little of it was reduced to moderately fine powder in a mortar and placed in the sun until thoroughly dry. In the meanwhile, two *burettes* were fixed on to a stand, the lower portion or point of one being connected to that of the other by means of a piece of India-rubber tubing supplied with a clip, so as to be able at will to interrupt the connection between the two tubes. A given quantity of soil (100 cubic centimeters) was carefully placed in one *burette*, and a similar amount of water in the other. The latter was allowed to flow into the former, which, as it ascended in the tube

Cholera said to affect some barracks more than others.

Method of ascertaining the porosity of soils.

containing the soil, was seen to drive out the air existing in the interstices, the amount of air displaced corresponding to the amount of water which entered. When the water came up to the upper edge of the soil in the tube, the connection between the tubes was interrupted, and the amount of water used read off.

Result of experiments.

As a few of the particulars of these observations may be useful for comparison when more exact data shall have been obtained of the relative liability of the barracks in question to cholera, I append them in a tabulated form :—

	Soil at a depth of 4 feet from	Amount of *air* contained in 100 parts by measure.	Permanganate solution required to give a permanent tint to a solution of 1oz. soil, 10ozs. water requiring 4 decems of the same solution.
Allahabad.	Clydesdale Lines, No. 8, south end ...	50·	5 decems
	„ „ „ 8, north „ ...	46·4	4 do.
	„ „ „ 3, south „ ...	53·3	8 do.
	„ „ „ 3, north „ ...	50·	... ..
	Chatham Lines, No. 8	50·	5 decems
	Artillery Lines, No. 2	50·	4 do.
	Wellington Lines	46·4	6 do.
	New Cantonment Barrack, No. 3 ...	46·4	4 do.
	Jail...	53·3	5 do.

Chemical examination of the soil.

Six of the specimens were subjected to chemical examination with the view of ascertaining whether the soil near the barracks, at or about three feet from the surface, contained an unusual amount of organic matter or not. One ounce of soil was taken and allowed to stand for twelve hours in pure water, shaking it a few times during this interval; it was then filtered, and the clear solution examined in the manner usually adopted for the examination of water.

The results were pretty much the same in all cases; except in those where the soil had been a "made" one, the amount of lime-salts varied, but I was surprised to find that the soluble organic matter, as estimated by a standard solution of *permanganate of potash*, did not much exceed the amount present in the ordinary drinking water of Calcutta when estimated by the same solution. (The exact relative amount of organic matter present may be ascertained by reference to the foregoing table). From these observations, therefore, I infer that in the ground beneath and about the barracks at Allahabad, both in the old *and* new cantonments, the amount of oxidisable matter was not in excess, at a comparatively short distance from the surface, at the time when cholera visited that station; consequently the epidemic could not have been owing to putrefying matter in the soil of the cantonments, unless such matter had been washed into the wells by the rain, and thus infected nearly a hundred and fifty persons belonging to the European troops stationed there. To have produced this, the amount of surface pollution present before the rains set in must, I should imagine, have been very extensive indeed.

Amount of organic matter in the soil was not excessive.

Inference.

Microscopic examination of the soil.

It was also thought desirable that a few samples of the earth should be taken and moistened with water in order to ascertain whether any special form of life, animal or vegetable, would make its appearance. I select two examples. A small portion of dry-earth from the new cantonment was placed in a test tube, to which a little water was added, sufficient to cover it. During the first and second days no particular forms of life were observed, but on the third and succeeding days several minute infusoria had become revived, and presented exceedingly active movements (Fig. xc).

On the third day ordinary infusoria appeared.

A similar portion of soil from the Clydesdale Lines was treated in the same way.

In another sample the animalculæ found in evacuations were plentiful.

In it also no particular objects were manifest for the first two or three days, but towards the end of a week, in addition to the objects delineated in the last figure, bodies in the circular, still, and active condition—not in any way distinguishable from the animalculæ already described as occurring in choleraic and other discharges—were seen to have developed in great numbers, some freely moving in the fluid, and others imbedded in granular matter (Fig. xci). Nothing further was observed in any of the samples, nor could I detect any evidence of the existence of the ultimate elements of fungi.

Visited the cholera-camps, the native portion of the city and surrounding villages.

I also accompanied the Sanitary Commissioner to the "cholera-camps" occupied by the 58th Regiment, about fifty miles from Allahabad, on the Jubbulpore road; and Dr. Chalmers, the Deputy Inspector General of Hospitals, very kindly undertook to show me nearly every part of the city and cantonment. To Dr. Irving also I am indebted for similar help. These excursions were undertaken more with the intention of getting a fair insight into the geography of the place, than of ascertaining what the exact sanitary arrangements were,—to report upon which not being the object of my visit. Careful notes, however, have been taken of what was seen and heard concerning the outbreak of the epidemic, but their narration would unnecessarily prolong this report and answer no good purpose. I hope, however, on a future occasion to turn what I then learnt to account.

CAWNPORE.

Visit to Cawnpore.

On the way to Lucknow, a few days were spent at Cawnpore. Compared to Allahabad, the troops in this station had suffered very little. Dr. Bryden states that the admissions were 27 and the deaths 17.

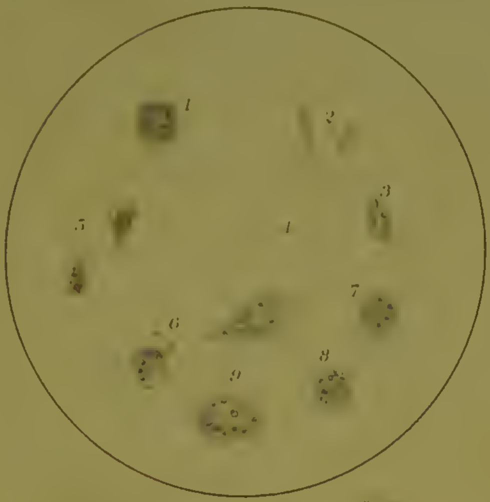
General nature of the soil.

The soil at Cawnpore is very like what it is at Allahabad, but contains less kunkur.

Near the artillery barrack there was more clay than elsewhere, but, as a rule, the ground is very permeable to water.

Wells, and their relation to the river.

Many of the wells are very much nearer the surface, water being found at five or ten feet, instead of fifty or sixty as at Allahabad. Nevertheless, some of the wells examined were thirty feet below the surface. Such a variation I did not observe at Allahabad. The ground slopes towards the Ganges: I could not ascertain whether the rise or fall in the river affected the level of the water in the station; but when the river rises, it swamps a large portion of the country along its banks.



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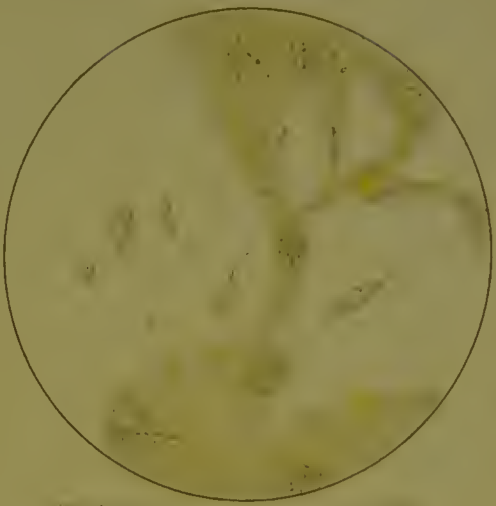

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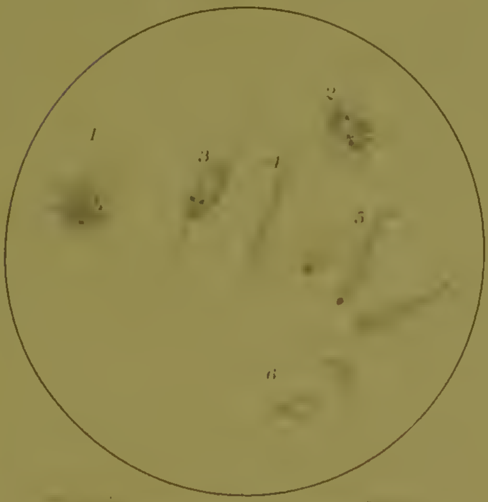

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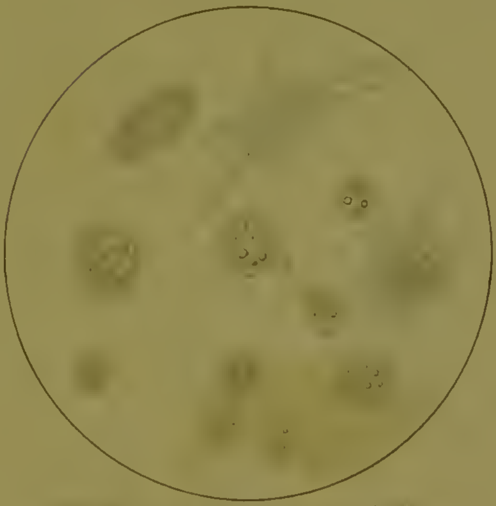

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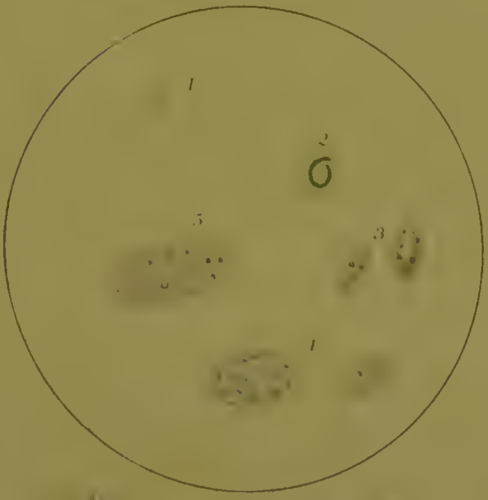

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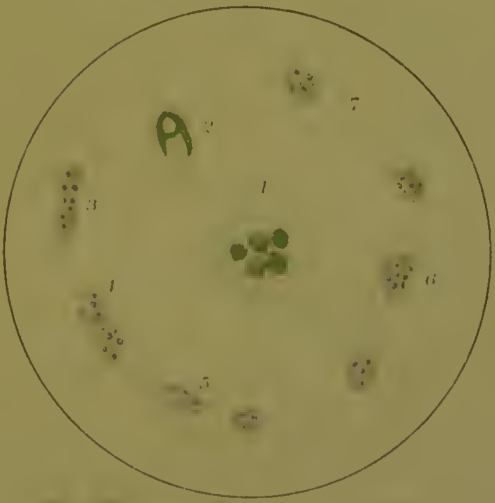

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FIGS XC I DEVELOPED IN MOISTENED SOIL FROM ALLAHABAD
XCII III LUCKNOW
XCIV FYZABAD
XCV MEERUT

Cholera-camp.

The cholera-camp of the 14th Regiment was pitched at Bhowpore. The ground about this camp was more sandy even than at Cawnpore, and more permeable to water and air. The relative degrees of permeability of this soil and of the soil near the barracks occupied by artillery, cavalry, and infantry regiments are given below :—

	Soil at a depth of 4 feet from	Amount of *air* contained in 100 parts by measure of soil.	Permanganate solution required to give a permanent tint to a solution of 1oz. soil, 10ozs. water requiring ·4 decems of the same solution.
Cawnpore.	Cholera-camp, Bhowpore	53·3	6 decems
	„ Residency	46·6	4 „
	Lines occupied by 19th Hussars	46·6	4 „
	„ „ Royal Artillery	46·6	4 „
	„ „ 14th Regiment	50·0	4 „

Organic matter.

Concerning the amount of organic matter in the soil, the same remarks will apply to this as were made relative to the soil at Allahabad. The soil from the camp at Bhowpore contained more than any of the others.

LUCKNOW.

Cholera mortality among the troops.

The European troops at this station suffered very severely from cholera, nearly a hundred deaths having taken place during the month of August, the men who had newly arrived from England, or had only lately been brought down from the hills, contributing most largely to swell this number.

Lucknow intersected by ravines.

Whilst visiting the various parts of this city, one could but note the extent to which it is intersected by ravines or *nullahs*, a faint conception of which may be obtained by observing the shaded portion of the accompanying little map, as well as of the swampy nature of the surrounding country. Some of these ravines are very deep and contained filth, others contained water which flowed into the Goomtee.

Much valuable information was obtained from Dr. Sutherland, the Sanitary Commissioner for Oude, concerning the course of the epidemic, which he had carefully noted on the spot; nevertheless, no clue could be obtained as to the origin of the cause of this mortality, or the mode by which it spread. In some cases the disease seemed to be localized to a particular spot, but in others no indication of such localization could be traced. As an example of the former, the following will aptly serve:—

Illustration of localization of the disease.

A man was seized with cholera in a barrack on the ground floor, and rapidly succumbed. The bed and bedding was removed and another replaced, which was occupied that night by another man, who was apparently perfectly well; he also sickened and died the same night! Another: a case occurred in the jail of a man who for a long time previously was not known to have been in communication with a single person from outside. His food and drink were precisely similar to the food and drink of the other prisoners. He was suddenly seized with cholera, and death resulted in a very short time, but the disease did not spread in the jail.

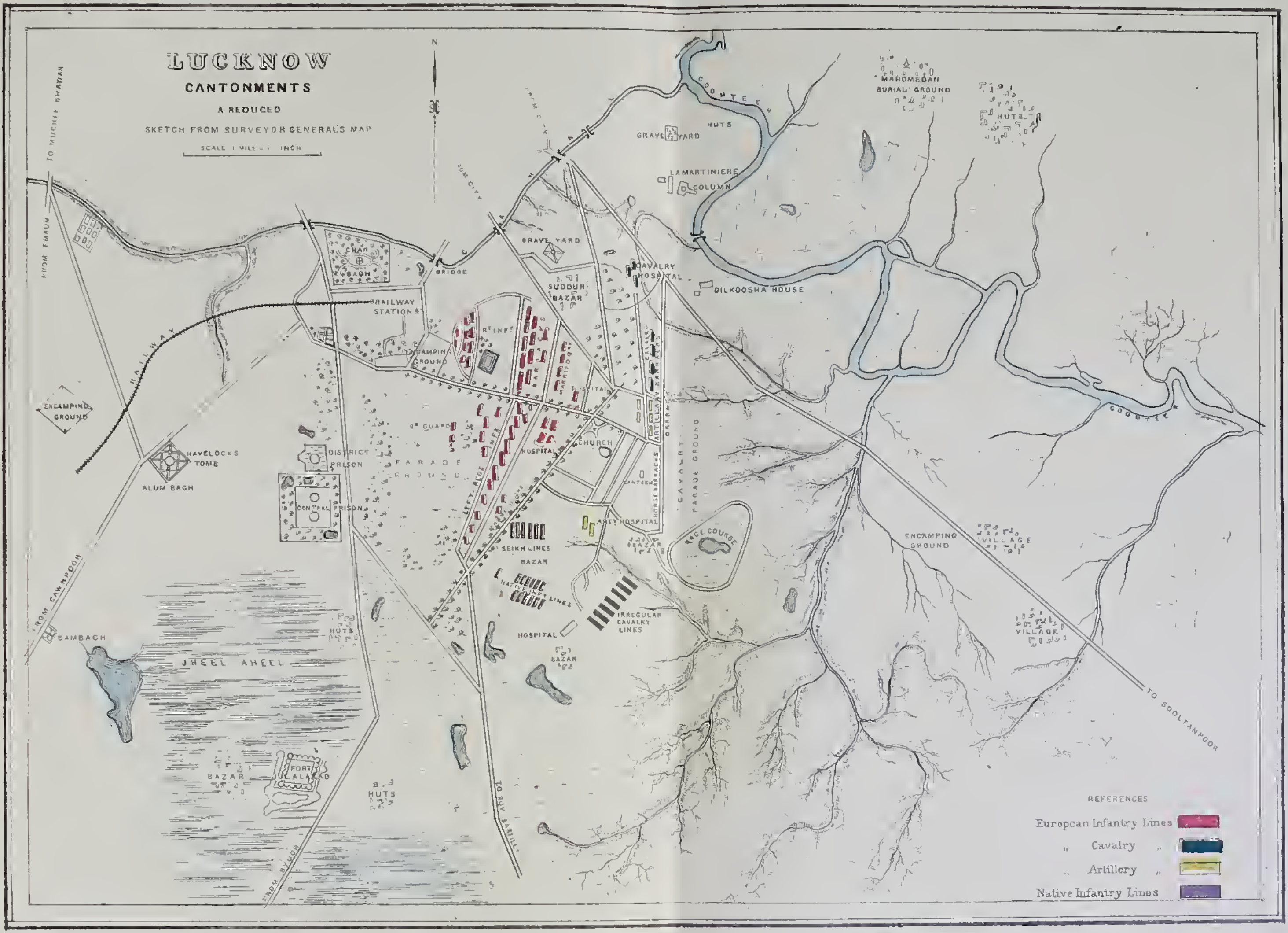
A single case in the jail.

What was the nature of the ground above which these persons lived? No difference could be detected between these and other places in this respect. The upper two or three feet consisted of rubbish, which had been used for "filling up;" then came a layer of sandy soil from two to three feet deep, which was quite moist, below which was a thin stratum of yellowish clay not sufficiently impermeable so as to be capable of holding water for any length of time, the permanent water-level being about thirty feet from the surface. This is attained by digging through some twenty feet of a white sandy soil. Speaking in general terms, this description will apply to the whole of the soil upon which Lucknow stands. It contains considerably more clay than exists in the stations already described, and was subsequently ascertained to be of a rather more impermeable nature.

Nature of the soil.

Extent of permeability and amount of organic matter.

It contained, however, in most places more organic matter, and the specific gravity of its solution was higher.



Lithographed in Colors at the Surveyor General's Office, Calcutta, August 1870.

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	Soil obtained from	Depth.	Amount of *air* contained in 100 parts by measure.	Permanganate solution required to give a permanent tint to a solution of 1oz. soil, 10ozs. water requiring 4 decems of the same solution.
		Feet.		
Lucknow.	No. 4 Barrack, occupied by Royal Artillery ...	3	50·	6 decems
	„ 2 „ „ „ „ ...	3	53·3	10 „
	„ 2 „ „ „ „ ...	6	53·3	10 „
	Hospital „ „ „ ...	3	53·3	8 „
	„ „ „ „ ...	6	53·3	...
	No. 12 Barrack, occupied by 62nd	3	53·3	16 „
	„ 12 „ „ „	6	50·	...
	„ 2 „ „ „	3	50·	12 „
	„ 2 „ „ by 102nd	3	53·3	12 „
	„ 4 „ „ „	3	50·	5 „
	„ 6 „ „ „	3	50·	14 „
	„ 3, Married Quarters, 5th Lancers ...	3	50·	...
	„ 2 „ „ „	3	46·6	6 „
	Jail, No. 7 building	3	50·	5 „
	„ „ „	6	50·	...

Microscopical examination.

Several specimens of soil were examined microscopically, but nothing could be detected in the moistened soil for the first two or three days, presenting unmistakable evidence of vitality. Infusorial animalcules of many kinds gradually appeared, but I could not state that any marked differences existed in the various specimens observed. A figure of those which revived in some soil, from No. 2 married quarters of the 5th Lancers, will serve to illustrate what these were (Fig. xcii).

In one sample, however, some very interesting low forms of life appeared, about which Mr. Huxley and Häckel have lately written so much. The test tube in which this particular sample (from a depth of six feet in No. 2 barrack, occupied by Royal Artillery) was seen was fortunately a very thin one, and permitted the use of a high power when placed on the stage of the microscope. The bodies observed consisted of minute masses of translucent, colourless jelly, without nucleus or contractile vesicle; in short, not the slightest evidence of structure existed. Their movements were very slow, slower than ordinary amœbæ, and being translucent, it was only by careful illumination that they could be watched. Two of them are sketched at Figure xciii, in the act of protruding long processes of their substance among some animalculæ which have become encysted on the walls of the tube. This moving substance presents precisely the same microscopical appearances as the hyaline, glary matter surrounding the encysted bodies. The little colony depicted of the latter was watched for several weeks, but no changes took place, consequently the nature of the encysted bodies could not be made out. It is very remarkable that such bodies retain their vitality so long, as they must have imbedded in this dry soil for several years.

A low form of life, corresponding to the *Monera* described by Hackel.

FYZABAD.

There were a few cases of cholera in this station also, but fortunately only two deaths occurred among the European soldiers. The cantonment is situated on slightly elevated ground on the banks of the Gogra, but no part of it is swamped by this river, nor is it believed that the rise and fall of the river affects the condition of the wells.

Site of cantonment at Fyzabad.

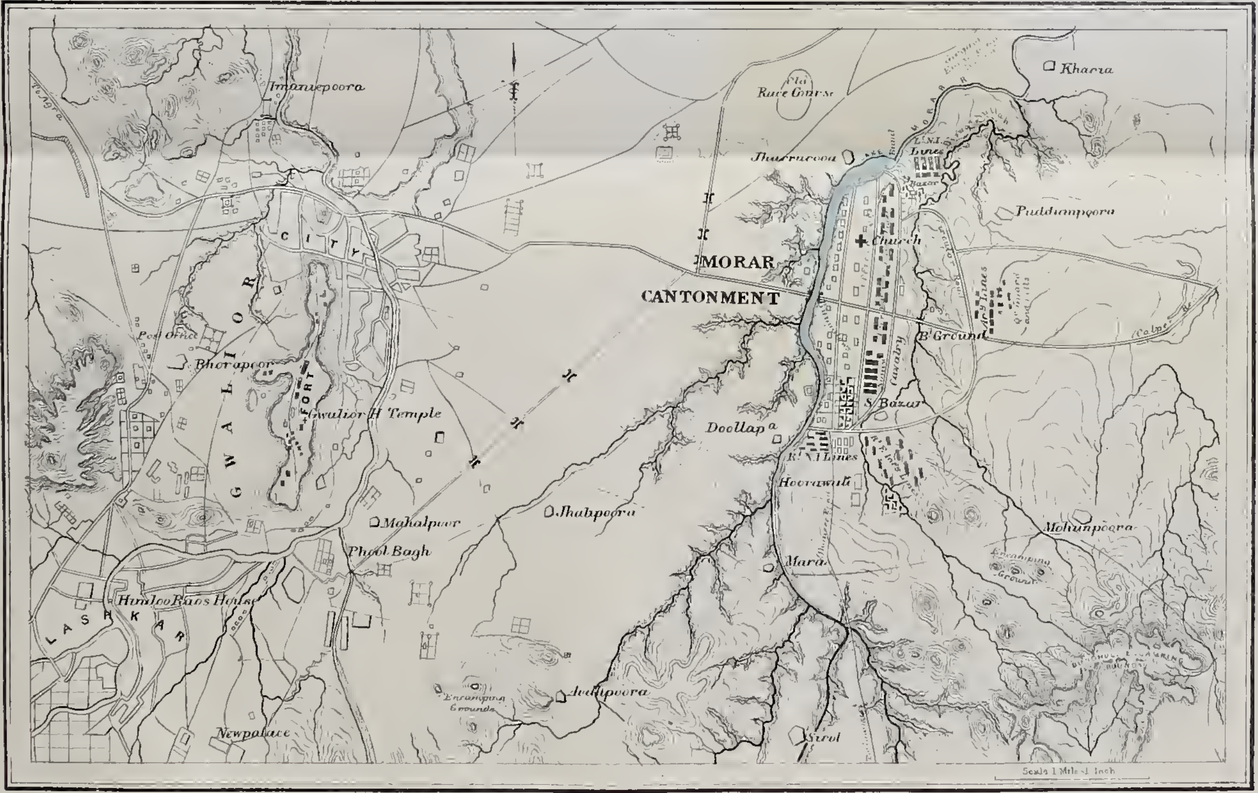
Relation of the river to the wells.

The soil is sandy everywhere, except near the bed of the river, where there are more traces of clay. Here and there a layer of kunkur is interposed between the upper more clayey layer and the lower one.

A few samples of the soil were preserved for subsequent examination, the result of which may be seen in the table below.

Examination of the soil.

On being microscopically examined, nothing which could possibly be construed as having the most remote connection with cholera could be seen. No spores of fungi could be identified, and the infusoria which became revived in the course of a few days were of the ordinary kind (Fig. xciv).



GWALIOR FORTRESS AND MORAR

A PORTION OF SURVEYOR GENERAL'S MAP.

ortality at Morar and alior Fort.

occurred among the European troops, although it was formerly considered one of the places exempted from demics of cholera. They had suffered severely in April, l still more so in August, sunstroke having been exceed-gly prevalent among the native population as well as olera. Every effort was made to get all the information ssible concerning the epidemic, more especially relating those points which seemed to bear upon the question to whether or not the origin and spread of the disease had y connection with the ground upon which the people stood.

elation of cantonment iver.

The cantonment is situate on a low-lying plain, surrounded by numerous hills on three sides, with the river Morar on the other. Some of the barracks are situ-ed below the level of the river, so that the drains have to taken in another direction. Other barracks, such as the es allotted to the Artillery, are about seventeen feet above e level of the Morar.

he artificial lake.

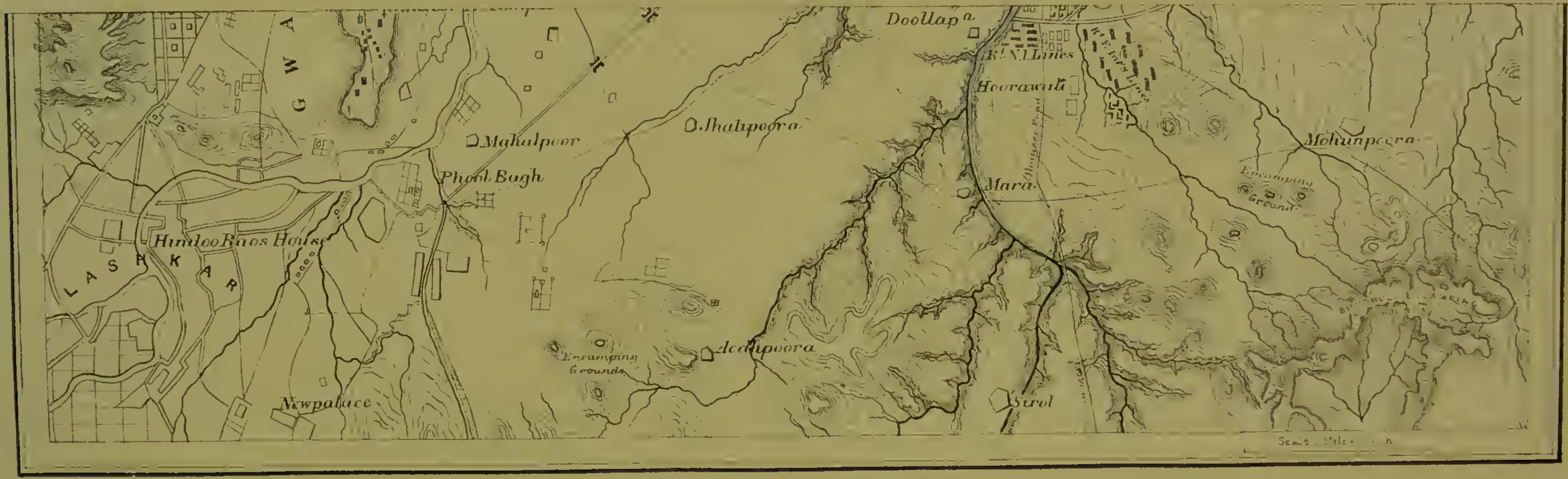
An embankment has been erected across the bed of e river, so as to provide the station with a sheet of "ornamental water," about a quarter of a mile at its widest part, and increas-g the depth of the river for about two miles above the m. It is not improbable that Dr. Pettenkofer, had he en here, would have made minute inquiries as to the tent of moisture supplied to the neighbouring subsoil by is artificial lake.

epth and variation of lls.

uality of water.

lack and red soil

The wells are from twenty to forty feet deep; the variation is said to be about five. The water is considered to be good. Dr. Whitwell has examined it very tely, and has kindly favoured me with the particulars of the analysis of an average sample; in this there is not a large amount of ganic matter, and no excess of deleterious salts. There are o kinds of soil at Morar, the *red* and *black* soil; both ntain persalts of iron, with lime and magnesia, but no *nitrates* nor *nitrites*, as one would have expected to find, had the ground een tainted to any great extent by the ordure of other ys; nor was the amount of oxidisable matter, as ascer-



GWALIOR FORTRESS AND MORAR

A PORTION OF SURVEYOR GENERAL'S MAP.

excessive, indicating that the barracks and their surroundings had not recently been subjected to contamination.

not regularly distributed;

The "black soil" was not universally distributed over the surface. Many yards of excavations were examined in which not a trace of this kind of soil existed; in others, again, a stratum of it was seen extending for long distances; at one end the layer might be ten feet, or more, in thickness, gradually diminishing until it was finally lost in the red; below these, a gritty sandy layer exists in which water is found. The foundation of several blocks of buildings, which were about being erected, were seen to present this uneven distribution of black and red soil, consequently the floorings of such buildings will vary in the extent to which they are permeable to gases, &c., from below; because the porosity of the red earth is considerably greater than that of the black. If Pettenkofer's theory be true, a building placed on this black clayey soil ought to be in a better sanitary condition than those built on the red—other things being equal. The relative porosity and amount of organic matter may be ascertained by reference to the table at the end of this paragraph. The samples enumerated are only a few of the ones examined, General Vaughan having most kindly procured specimens from every portion of the cantonment.

hence a barrack may stand on soils of varying porosity.

Cholera-camps.

The cholera-camp was four or five miles out of the station, near the summit of two or three little rocky hills, the hospital apparently having a little hill for itself.

Fortress of Gwalior.

The *Fortress of Gwalior* is about six miles to the west of the cantonment of Morar. It stands on a rock whose summit is about 1¾ mile in length and about ½ mile across in its widest part, and from 300 to 400 feet high, the ascent to which is very steep. Immense fissures may be observed in the rock whilst ascending the steep towards the gate at the entrance of the fort, these being for the most part filled with earth. On entering the fortress, nothing is seen but huge blocks of buildings standing on a barren rock strewn with a few half-withered trees, or rather shrubs. The surface of the rock is naturally very uneven, stone forming the foundation of one end of a building, whilst frequently "made" soil, to the depth of twenty or thirty feet,

A barren-fissured rock

forms the foundation of the other. The rock itself is a sandstone; splits to any extent, and very easily worked when wet, but excessively hard when dry. It is porous to the extent of one-third of its bulk, consequently able to retain a great amount of any sewage that may be thrown upon it.

of sandstone.

Temperature.

The heat on this rock is very great; it is much complained of, especially as it continues during nearly the whole night, because by the time that it begins to cool, the rays of the sun are directed towards it again.

No original soil.

The samples of soil obtained consisted entirely of rubbish; there does not seem to be an inch in the place undisturbed by man until the bare rock is attained.

Table showing relative porosity and organic matter in the soil.

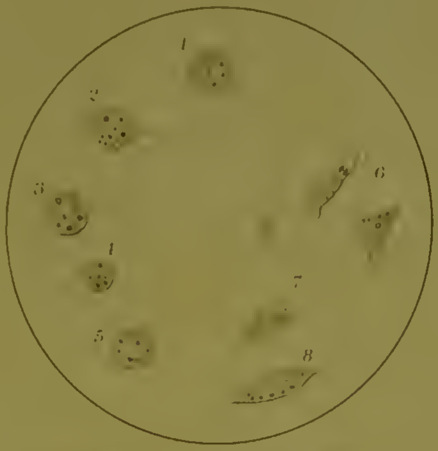
	Soil from	Depth.	Amount of *air* in 100 parts of soil by measure.	Permanganate solution required to give a permanent tint to 1oz. soil, 10ozs. water requiring 4 decems of same solution.
Morar.	(*Red.*)			
	No. 4 Barrack, occupied by R. A.	3	46·6	6 decems
	Ditto ditto	6	46·6	9 „
	No. 9 Barrack, occupied by 103rd	3	66·	6 „
	No. 2 Barrack, ditto	3	..	5 „
	No. 4 Barrack, occupied by R. A. (Yellow clay)	3	45·	4 „
	(*Black.*)			
	No. 2 Barrack, occupied by R. A.	3	31·	5 „
	Ditto ditto	6	36·	4 „
	No. 6 Barrack, occupied by Md. Qrs., R. A.	...	33·	6 „

MEERUT.

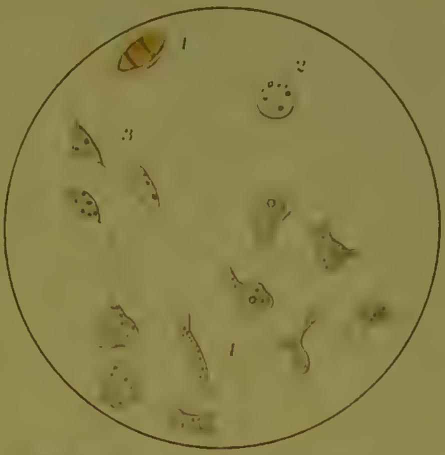
The epidemic at Meerut preceded by rain.

Cholera visited this station in September and the beginning of October, having been preceded by a heavy fall of rain. Nineteen cases occurred, with fourteen deaths, among the European soldiers, whilst about a hundred cases occurred among the natives of the bazaar. The cantonment is situated on a large plain, with scarcely any fall, consequently not admitting of good natural drainage. There is a deep ravine separating the European and the Native lines, on either side of which for a short distance good clay is found; otherwise it is rather sandy everywhere, quicksand being frequently met with in digging the foundation of a building.

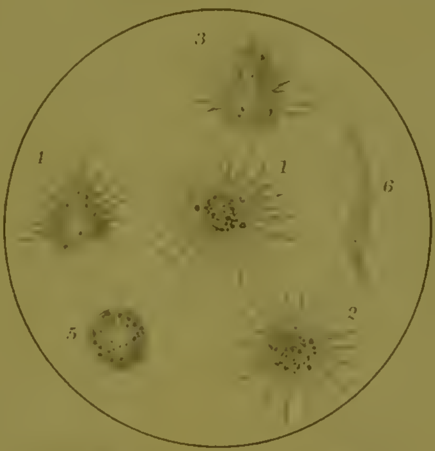
Nature of soil.



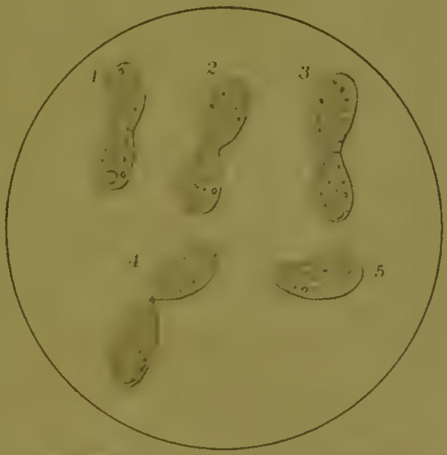
1 6 [illegible]
7 8 [illegible]



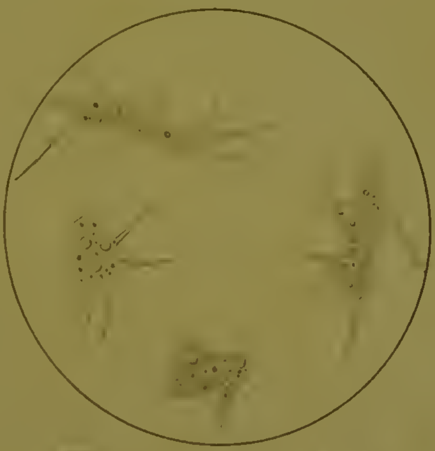
1. [illegible] 2 3 [illegible]
4. [illegible]



[illegible] 1 5
6 [illegible]



1 4 [illegible]
5 [illegible]



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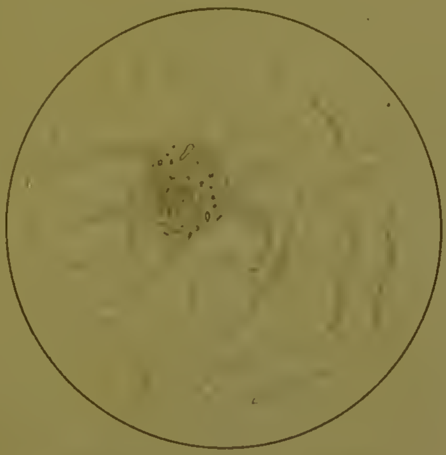

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FIG XCVI DEVELOPED IN MOISTENED SOIL FROM MEERUT
FIGS XCVII CI PESHAWUR

The wells are not very deep, water being generally attained at about 10 to 12 feet from the surface, the extreme variation in which is, according to Dr. Berkeley, about five feet. Rain rapidly affects the level of the water in the wells, the amount of rise of the latter being almost equal to the fall of the former. This is the reverse of what occurs at Allahabad, where a great portion of the rainfall either drains to the river or is evaporated before reaching the permanent water-level. This intimate connection between the wells and the surface at Meerut is of great sanitary importance. Seeing the ease with which any sewage may get into the wells, and as the condition of the ground does not permit of free natural drainage, it is self-evident that the greatest attention should be paid to remedying this defect by artificial means.

Intimate connection between surface drainage and the wells.

In the more minute examination of this soil, subsequently undertaken, no evidence existed of the ground in the vicinity of the barracks being in a polluted condition, and on the whole was rather less porous than the soils already alluded to, with the exception of the black soil at Morar.

Relative amount of organic matter and porosity of the soil.

	Soil at a depth of 4 feet from	Amount of *air* contained in 100 parts by measure.	Permanganate solution required to give a permanent tint to a solution of 1oz. soil; 10 ozs. water requiring 4 decems of some solution.
Meerut...	Between Nos. 44 & 46 (105th Regiment).	45·	5 decems
	„ „ 34 & 39 „ „ ...	50·	6 „
	„ „ 43 & 48 „ „ ...	50·	5 „
	„ „ 1 & 2 (4th Hussars) ...	55·	5 „
	„ „ 13 & 14 „ „ ..	46·3	5 „
	Married Quarters, No. 15, R. A.	50·	6 „

This soil was examined microscopically in the same manner as the others were, with somewhat similar results. During the first few days its solution contained no infusoria, at least not in motion, but subsequently they made their appearance in great numbers. These in one sample, namely, Microscopical examination of soil.

in the soil from between Nos. 1 and 2 blocks, occupied by the 4th Hussars, consisted almost entirely of various phases in the existence of *monas lens* (Figs. xcv & xcvi). These alter their form very rapidly, frequently protruding an amœba-like vesicle, as seen at Nos. 1 to 5, Figure xcv, which represents one animalcule assuming different forms. There are also great numbers of very minute amœbæ (6) which seem to be an earlier stage of this animalcule, and when it gets older it becomes elongated (7-8), sometimes acquiring two filaments. They are frequently seen to multiply by division, as seen in Figure xcvi, where No. 3 runs through the stages delineated at 4 to 7 in the course of five minutes, the two at 7 becoming as perfect in all points as the original one. The green bodies in the figures, which rolled about the field, are *algæ*.

PESHAWUR.

Soil from Peshawur.

The Sanitary Commissioner having subsequently visited Peshawur (where over 350 cases of cholera were reported as having occurred during the month of September among the European troops alone), favoured me with two samples of soil, one sample from a depth of three feet, and the other from a depth of six.

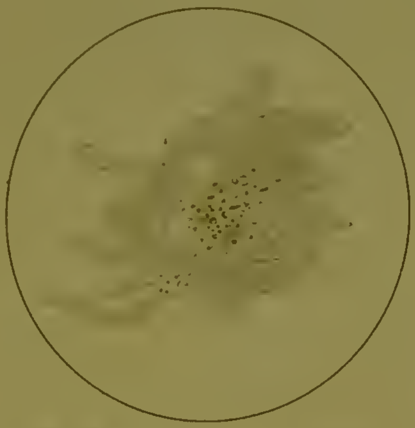
Spongy nature of soil.

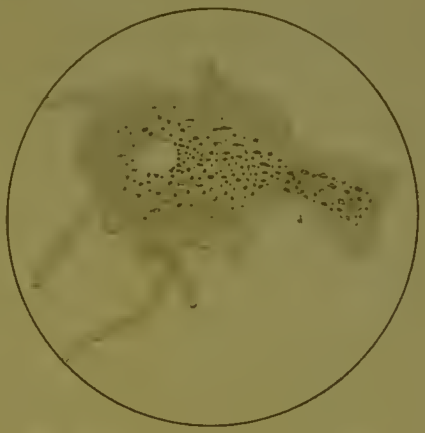
It was in hard lumps, of low specific gravity, owing to its spongy nature, exceedingly like a piece of pumice stone, and when applied to the lips, so freely could air be made to pass through, that a feather placed on one end of a table could be readily blown to the other.

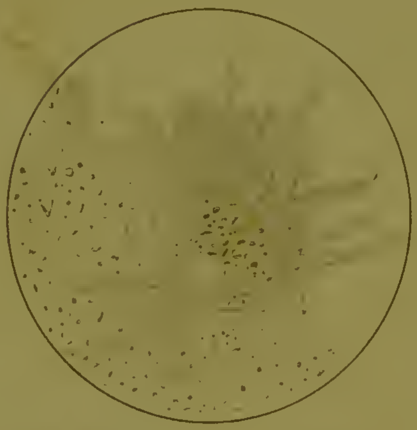
Its relative porosity and organic contents.

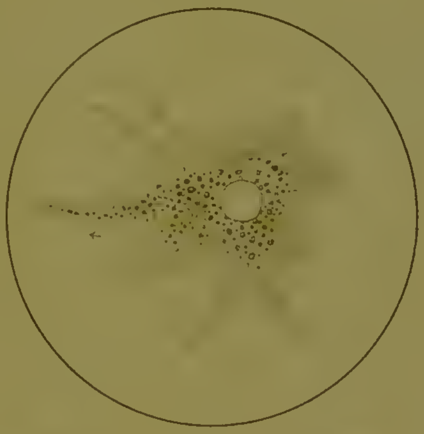
Its solution was slightly alkaline, and contained rather more organic matter than the average, as may be seen from the subjoined table.

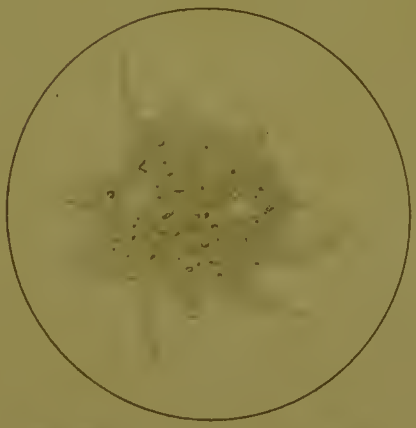
	Soil from a depth of	Amount of *air* in 100 parts by measure.	Permanganate solution required to give a permanent tint to a solution of 1oz. soil, 10ozs. water requiring 1 decems of same solution.
	Feet.		
Peshawur.	3	50·	10 decems
	6	50·	8 ..

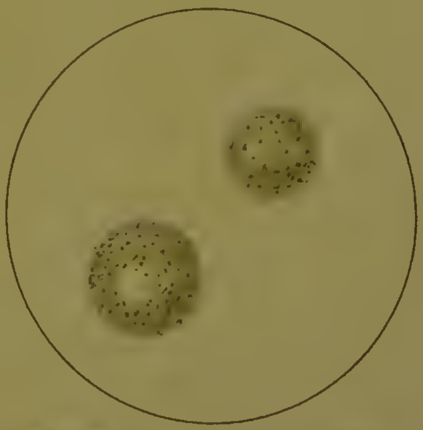












FIGS CII CVI DEVELOPED IN MOISTENED SOIL FROM PESHAWAR
VARIOUS FORMS ASSUMED BY ONE IN TWO MINUTES
FIG CVII FOREGOING BECOME SPHERICAL & STILL

Microscopic examination.

It was subjected to a prolonged microscopic examination. During the first three days a number of molecules developed in the fluid containing the three feet soil; then an abundance of animalcules like the ones alluded to in connection with the Meerut soil (Fig. xcvii, 2-3). At Nos. 5, 6, and 7 various forms are depicted, assumed by one in two minutes, which was also occasionally seen to jerk suddenly in the same manner as 2 and 3. The reddish body at 1 is a spore, probably belonging to the *Dematiei* family—a very common fungus.

Another of Häckel's *Moners* (?)

The test tube containing the other sample of soil from a depth of six feet having been left undisturbed for a week, was, on examination, found to contain several examples of slimy bodies of a lower organization than the amœba, there being no contractile vesicle, although generally one or more vacuoles were seen (c). Nearly all of them contain molecular matter, which flows towards the portion of substance in the act of being projected. Figures cii to cvi illustrate the various forms assumed by one of these in the course of twenty minutes. They were not seen to divide, nor did the protruded processes become amalgamated when they crossed each other. A great number of vibriones developed in this solution, more so than I had observed in any of the other specimens of soil examined, and were very active. These are figured at ci, amongst which one of the just described *moners* is seen with extended processes, which were observed to wander throughout the fluid something like the "horns" of a snail. To these processes monads and small vibriones adhered, which were drawn into the substance of the *moner* as the processes were retracted. Three days afterwards, all the *moners* had become spherical and perfectly still (cvii).

Vibriones unusually prevalent.

Monera become spherical.

The other animalcules which made their appearance were those commonly met with, and require no special description. They are figured at xcviii and xcix, where the names are also given.

Concluding remarks.

Having already alluded to the chief points in connection with these experiments, whilst describing the various places visited, it is not considered necessary to refer to them again. The observations concerning the physical geography of the stations are of a more superficial nature than I could have desired, but the time at my disposal was very limited, and correct information on such matters could not be obtained without personal inspection. It will, indeed, be evident that the experiments referred to in the whole of this report are of an elementary nature. This is, in part, owing to the short period which has elapsed since they were commenced, partly also to my having been tempted, by the desire for results, to keep too many irons in the fire. I trust, however, that what has been done will prove to be a foundation whereupon better things may be built.

In conclusion, I respectfully tender my most sincere thanks to Dr. Muir, C. B., Inspector General of Hospitals, British Troops, for the assistance which he has so gladly rendered on every possible occasion to further this inquiry, and for the personal interest he has taken in the details thereof; also to Dr. Cuningham, the Sanitary Commissioner with the Government of India, for similar aid, not less cheerfully given.